2001 Project Abstract FINAL REPORT AUG 18 2004

For the Period Ending June 30, 2004

TITLE:	Biological Control of Eurasian Watermilfoil and Purple Loosestrife-Continuation
PROJECT MANAGER:	Luke Skinner
ORGANIZATION:	Minnesota Department of Natural Resources
ADDRESS:	500 Lafayette Road, Box 25, St. Paul, MN 55155-4025
FUND:	Environment and Natural Resources Trust Fund
LEGAL CITATION:	ML 2001, 1st Special Session, Chap. 2, Sec. 14, Subd. 04(d)

APPROPRIATION AMOUNT: $90,000

Overall Project Outcome and Results

The purpose of this research was to evaluate biological controls for Eurasian watermilfoil, *Myriophyllum spicatum*, and purple loosestrife, *Lythrum salicaria*, two exotic aquatic plants that are degrading Minnesota's aquatic resources statewide. Researchers found that the milfoil weevil, *Euhrychiopsis lecontei*, can cause sustained declines of the invasive, non-native Eurasian watermilfoil if sufficient densities of the insect are maintained throughout the summer each year. Unfortunately, in many lakes, weevils do not reach adequate densities, or their densities do not persist through the summer over several years, to sustain control. In many lakes, sunfish appear to limit densities of the milfoil weevil, and so prevent sustained declines in Eurasian watermilfoil. Also, sustained control of this non-native plant is likely to require an increase in rooted native plants following reductions in the amount of the invasive species. For a complete description of the Eurasian watermilfoil research, see Newman (2004).

Evaluation of purple loosestrife biological control found that the leaf-beetles, *Galerucella* spp., can provide long-term control of purple loosestrife. As purple loosestrife populations were reduced, the diversity of other plant species increased (Skinner et al.2004). *Galerucella* ssp. populations fluctuate over time in response to purple loosestrife abundance. At some sites, the leaf beetle populations declined and have not rebounded, suggesting control may vary depending on a number of factors *Galerucella* spp. did not impact two native Lythrum species. Although *Galerucella* larvae were present and some feeding observed on swamp and winged loosestrife, plant growth or reproductive parameters were not affected (Stamm Katovich et al. 2004). *Galerucella* spp. can readily disperse and colonize purple loosestrife infestations within wetlands and across landscapes. *Galerucella* spp. on average, dispersed 5 km to new purple loosestrife infestations within 3 years. The maximum dispersal distance recorded was 20 km. Beetles were found in 85% non-release sites visited (McCornack et al. 2004).

Project Results Use and Dissemination

Results of this project will be published in peer-reviewed scientific journals and also in special publications and newsletters. Results also will be presented at national, regional and state scientific meetings to peers in the field, as well as to resource managers and planners who will use the results of this project. Currently, the research results are used in decision making for management activities in the state. For example, recent results provide guidance for releasing purple loosestrife control agents and what to expect after release. A list of future publications can be found in the final report.

Date of Completion: August 18, 2004

LCMR Final Work Program Report

I. PROJECT TITLE: Biological Control of Eurasian Watermilfoil and Purple Loosestrife- Continuation

Project Manager: Luke Skinner
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Total Biennial Project Budget: **$90,000**

$ LCMR Appropriation $90,000 **$ Amount Spent**: $90,000 = **$ Balance:** $0

Legal Citation: ML 2001, 1st Special Session, Chap. 2, Sec. 14, Subd. 04(d)

Appropriation Language: $45,000 the first year and $45,000 the second are from the trust fund to the commissioner of natural resources for the fifth biennium of a five biennia project to develop and implement biological controls for Eurasian water milfoil and purple loosestrife. This appropriation is available until June 30, 2004, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.

II. FINAL PROJECT SUMMARY

The purpose of this research was to evaluate biological controls for Eurasian watermilfoil, *Myriophyllum spicatum*, and purple loosestrife, *Lythrum salicaria*, two exotic aquatic plants that are degrading Minnesota's aquatic resources statewide. Researchers found that the milfoil weevil, *Euhrychiopsis lecontei*, can cause sustained declines of the invasive, non-native Eurasian watermilfoil if sufficient densities of the insect are maintained throughout the summer each year. Unfortunately, in many lakes, weevils do not reach adequate densities, or their densities do not persist through the summer over several years, to sustain control. In many lakes, sunfish appear to limit densities of the milfoil weevil, and so prevent sustained declines in Eurasian watermilfoil. Also, sustained control of this non-native plant is likely to require an increase in rooted native plants following reductions in the amount of the invasive species. For a complete description of the Eurasian watermilfoil research, see Newman (2004).

Evaluation of purple loosestrife biological control found that the leaf-beetles, *Galerucella* spp., can provide long-term control of purple loosestrife. As purple loosestrife populations were reduced, the diversity of other plant species increased. *Galerucella* ssp. populations fluctuate over time in response to purple loosestrife abundance (Skinner et al.2004). At some sites, the leaf beetle populations declined and have not rebounded, suggesting control may vary depending on a number of factors *Galerucella* spp. did not impact two native Lythrum species. Although *Galerucella* larvae were present and some feeding observed on swamp and winged loosestrife, plant growth or reproductive parameters were not affected (Stamm Katovich et al. 2004). *Galerucella* spp. can readily disperse and colonize purple loosestrife infestations within wetlands and across landscapes. *Galerucella* spp. on average, dispersed 5 km to new purple loosestrife infestations within 3 years. The maximum dispersal distance recorded was 20 km. Beetles were found in 85% non-release sites visited (McCornack et al. 2004).

IV. OUTLINE OF PROJECT RESULTS

Detailed descriptions of the background for each objective listed below, as well as proposed methods to accomplish these objectives, are provided in two proposals written by the researchers who will do this work. The proposals are included as attachments B1 and B2 to the workprogram.

A. Eurasian watermilfoil

Result 1. Research on the potential for biological control of milfoil is subdivided into three activities, which are described below.

Activity 1. Attempt to detect additional lake-wide milfoil declines and assess populations of the milfoil weevil in a broader array of lakes. Continue to sample intensive sites and begin to sample six new lakes with a range in densities of sunfish. These tasks will allow us to determine if milfoil declines are occurring and if declines are related to control agent occurrence or densities. These results will be combined with information in task B to determine if sunfish or other factors may be limiting weevil densities.

LCMR Budget: **$5,000** Other: $7,500
Balance: $ 0 Other Balance: $ 0
Completion Date: June 30, 2004

Activity Status: Intensive monitoring of five lakes documented declines of Eurasian watermilfoil in four of these lakes. These declines appear to be related to herbivory by biological control agents. Two declines were lake-wide and persisted for 3 or more years. Observations by this team of researchers from the University of Minnesota and work from elsewhere indicates that milfoil weevils can control Eurasian watermilfoil when adequate densities of weevil are reached and sustained. Nevertheless, in many lakes, weevils do not reach adequate densities or their densities do not persist through the summer over several years to sustain control. For a complete description of the research done under this activity, please see Newman (2004).

Activity 2. Identify and manipulate factors that limit populations of milfoil biocontrol agents such as the milfoil weevil. Continue biweekly surveys of weevil densities in four lakes. The results of this task and the lakewide data from task A will allow us to determine if weevil density and longevity are related to sunfish density and other lakewide factors. Conduct large-scale open augmentations of weevils in two lakes, one with high sunfish and the other with low sunfish. This task will allow us to further assess the importance of sunfish on the establishment and success of the milfoil weevil.

LCMR Budget:: **$20,000** Other: $20,000
Balance: $ 0 Other Balance:$ 0
Completion Date: June 30, 2004
Activity Status: Regressions suggested that sunfish density explains 60 and 70% of the variation in total weevil and adult weevil density, respectively, among lakes. This result supports experimental observations by researchers from the University of Minnesota that sunfish predation is an important factor limiting weevil density, and thus milfoil control, in Minnesota lakes.

Though stocking of two study lakes resulted in establishment of detectible weevils populations that carried over to the next summer, there were no significant reductions of milfoil associated with weevil stocking in either lake.

For a complete description of the research done under this activity, please see Newman (2004).

Activity 3. Identify and manipulate factors that may limit the effectiveness of milfoil control agents (plant community response). Assess the response of the plant community to the effects of treatment with alum on water clarity in Minneapolis lakes. Experimentally manipulate plant communities to determine the importance of plant competition on abundance of milfoil in one of the Minneapolis lakes treated with alum and in two other lakes with low water clarity. Determine if exchangeable N is an important factor in determining plant community composition, abundance of milfoil, and response of the plants to biocontrol agents.

LCMR Budget:: **$20,000** Other: $22,500
Balance: $ 0 Other Balance:$ 0
Completion Date: June 30, 2004

Activity Status: Attempts to increase water clarity via treatments with alum did not enhance native plant communities. In three Minneapolis lakes with successful alum treatments, Eurasian watermilfoil maintained or increased its dominance after treatment. It is possible that the increases in clarity were not sufficiently large, or sustained for a long enough time, to benefit native plants. Alternatively, a reduction in the abundance of milfoil, such as might be caused by herbivory, may be needed to reduce milfoil's competitive advantage and allow native plants to increase.

To test the hypothesis that competition among different plant species may affect reestablishment of Eurasian watermilfoil after a decline or reduction due to weevil damage, experimental removals of milfoil and other plants were conducted. Overall, the experimental removals did not reveal dramatic shifts or competitive interactions among plant species. Coontail tended to move into the plots from which milfoil was removed, but the milfoil recovered within a year. Somewhat surprisingly, milfoil did not increase rapidly in the plots from which all plants were removed. Milfoil appears to be slow to recover from removal due to its need to develop an extensive root system. The lack of increase in rooted native plant species enabled milfoil to again become dominant a year or more after removal.

This research found some support for McComas's (1999, 2003) hypothesis that native plants will do better than milfoil on low nitrogen sites, but milfoil will reach nuisance levels on high nitrogen sites. If milfoil is controlled by factors other than sediment, such as herbivory or water clarity, it will not reach nuisance levels, apparently even on high nitrogen sites. High levels of milfoil biomass appear less common on low nitrogen sediments.

For a complete description of the research done under this activity, please see Newman (2004).

Reports cited

McComas, S. 1999. The role of lake soils in managing lakes. Focus 10,000 11(1): 7-9.

McComas, S. 2003. Lake and pond management guidebook. Lewis Publishers, Boca Raton, FL.

Newman, R.M. 2004. Biological control of Eurasian watermilfoil: Completion report for 2001-2004. Unpublished report dated June and submitted by the Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota, Saint Paul, MN 55108 to C.H. Welling, Eurasian Watermilfoil Program, Division of Ecological Services, Minnesota Department of Natural Resources, 500 Lafayette Rd., Saint Paul, MN 55155.

B. Purple loosestrife

Result 2. Evaluate the effects Galerucella spp. on beneficial wetland plant community, divided in to 2 activities below.

Activity 1. Documentation of the beneficial wetland plant community response to release of *Galerucella* spp. for purple loosestrife control. The effect of *G. calmariensis* and *G. pusilla* on plant communities will be studied at a minimum of two locations. The objective of this study is to determine the impact of *Galerucella* leaf defoliation on purple loosestrife plants and nontarget wetland species abundance and diversity.

LCMR Budget: **$15,000** Other: $20,000
Balance: $ 0 Other Balance: $ 0
Completion Date: December 31, 2003

Activity Status:
Long-term monitoring projects that examine the effect of biological control agents on target weeds and native plants are important components of biological control projects. We examined ata collected by the Ecological Services Division of the Department of Natural Resources from 1997 through 2003 is currently being analyzed. Permanent transects were established in a variety of wetlands throughout Minnesota. The transects have been monitored on a yearly basis for the impact of *Galerucella* spp. on purple loosestrife and other native wetland plants. These data are currently being analyzed and results of this study will be submitted for publication in a peer-reviewed journal. Preliminary results suggest that significant declines are occurring in purple loosestirife populations in response to feeding by *Galerucella* spp. Native plants are also rebounding in several of the sites. Results will be submitted for publication in a peer-reviewed journal and for final LCMR report.

For a complete description of the research done under this activity, please see Skinner et al. (2004).

Activity 2. Assess impact of *Galerucella* spp. on *Lythrum alatum* and *Decodon verticillatus*. *Galerucella* spp. adult feeding and presence of egg masses have been reported on the non-target plants, winged and swamp loosestrife. However, the effect of *Galerucella* feeding on non-target plant growth and development has not been investigated. Phenology of *Galerucella* spp. emergence in the spring in relation to the emergence of winged and swamp loosestrife will also be evaluated.

LCMR Budget: **$10,000** Other: $10,000
Balance: $0 Other Balance: $0
Completion Date: December 31, 2003

Activity Status:

Previous studies have characterized the feeding, oviposition and larval development of the biological control insects, *Galerucella* spp., on non-target Lythraceae species, including two species native to Minnesota, winged loosestrife (*Lythrum alatum*) and swamp loosestrife (*Decodon verticillatus*). However, the impact of *Galerucella* spp. feeding on growth and seed production of the non-targets, winged loosestrife and swamp loosestrife, has not been reported. The objective of this study was to compare the phenology, growth and seed capsule production of winged loosestrife and swamp loosestrife, in relation to purple loosestrife (*Lythrum salicaria*), with and without the impact of *Galerucella* spp. Our study has documented minimal larval feeding on winged loosestrife and swamp loosestrife from the first generation of beetles in mid-June. Although *Galerucella* larvae were present on swamp and winged loosestrife, with one exception, none of the measured plant growth or reproductive parameters were reduced as a result of larval or adult *Galerucella* feeding. In the first year of the study, the number of winged loosestrife seed capsules were reduced with *Galerucella* feeding compared to control plants. However, there were no *Galerucella* spp. present on winged loosestrife in the second year of the study. In Minnesota, flowering and seed development in swamp loosestrife occurs a month later than in purple loosestrife or winged loosestrife. Since *Galerucella* larval shoot tip feeding reduces the number of seed capsules formed on purple loosestrife, missing the main period of larval feeding in mid-June provides a degree of "phenological protection" for swamp loosestrife from *Galerucella* spp. feeding.

For a complete description of the research done under this activity, please see Stamm-Katovich et al. (2004).

Result 3. Two studies will document movement of *Galerucella* spp. within wetlands and on a landscape scale (miles) where wetlands with purple loosestrife are spatially isolated. *Galerucella* will move through purple loosestrife infested wetlands where there is a A green bridge of purple loosestrife plants connecting two distinct areas of infestation. Circumstantial evidence indicates that mass movement occurs when small barriers, e.g., 50m of woods without loosestrife, separate two large areas of purple loosestrife (observations from Winona, MN August 2000). These studies will provide information that will help guide release strategies for loosestrife management in Minnesota.

LCMR Budget: **$20,000** Other: $20,000
Balance: $0 Other Balance: $ 0
<u>Completion Date</u>: December 31, 2003

Result Status:

In 1992, leaf beetles *Galerucella calmariensis* and *G. pusilla*, were introduced from Europe as biological control agents against purple loosestrife, *Lythrum salicaria* L. The ability of *Galerucella* spp. to control or reduce purple loosestrife infestations has been well documented. However, there is a limited knowledge regarding the ability of this insect to disperse, and a technique often used to study insect spatial distributions is geostatistics. The objectives of this study were to 1) characterize the spatial distribution of *Galerucella* spp. within a wetland and 2) evaluate the ability of *Galerucella* spp. to disperse to noncontiguous loosestrife infested wetlands on a landscape-scale. Our results suggest that *Galerucella* spp. can disperse and colonize purple loosestrife infestations within wetland habitats shortly (less than three years) after the initial release. In our experiment, apparent reductions in purple loosestrife infestations were often related to high egg mass densities of *Galerucella* spp. egg masses and beetle damage observed in the spring. This trend was present in all four wetlands studied. On a landscape level, *Galerucella* spp. appear to be well adapted to changing environments and are capable of dispersing and colonizing distant purple loosestrife infestations. On average, beetles dispersed 5 km from established release sites to non-release sites within 3 years. The average maximum dispersal distance from all four locations was approximately 19 km. Beetles were found in 85% of the 167 non-release sites visited. To maximize redistribution efforts of the biological control agents, we advise resource managers to select wetlands that are greater than 5 km from known release sites. *Galerucella* spp. is capable of colonizing new purple loosestrife infestations, thus reducing redistribution efforts from resource managers.

For a complete description of the research done under this activity, please see McCornack (2004).

Reports Cited

Stamm Katovich, E. J., R.L. Becker, D.W. Ragsdale and L.C. Skinner. 2004. Growth and phenology of three Lythraceae species in relation to *Galerucella* spp. Unpublished report submitted by the University of Minnesota, Saint Paul, MN 55108, to Luke Skinner, Purple Loosestrife Program, Division of Ecological Services, Minnesota Department of Natural Resources, 500 Lafayette Rd., Saint Paul, MN 55155. Final Report for Result 2, Activity 2.

McCornack, B. P., L.C. Skinner and D.W. Ragsdale. 2004. Landscape-Scale and Within Wetland Movement of *Galerucella* spp. Introduced for Management of Purple Loosestrife (*Lythrum salicaria* L.). Unpublished report submitted by the University of Minnesota, Saint Paul, MN 55108 to Luke Skinner, Purple Loosestrife Program, Division of Ecological Services, Minnesota Department of Natural Resources, 500 Lafayette Rd., Saint Paul, MN 55155. Final Report for Result 3 to Minnesota Department of Natural Resources.

Skinner, L.C., E.J. Stamm Katovich, D.W. Ragsdale, W.J. Crowell, N. Proulx and R.L. Becker. 2004. Population Dynamics and Long-term Effects of *Galerucella* spp. on Purple loosestrife, *Lythrum salicaria,* and non-target native plant communities in Minnesota. Unpublished report to the Legislative Commission on Minnesota Resources. Final Report for Result 2, Activity 1.

V. TOTAL PROJECT BUDGET:
All Results:
Other: $ 90,000 (Contracts with the University of Minnesota)

Total Budget: $90,000

VI. PAST, PRESENT AND FUTURE SPENDING:
A. Past and Current Spending:

	July 91-June 93	July 93-June 95	July 95-June 97	July 97-June 99	July 99-June 02	July 01-June 04
LCMR	$160,000	$400,000	$300,000	$150,000	$150,000	$90,000
Other state	--	--	--	$150,000	$150,000	$100,000
In-kind	--	$200,000	--	--	--	--
Total	$160,000	$400,000	$300,000	$300,000	$300,000	$190,000

C. Cooperation and Project Partners:

The DNR's Exotic Species Program applied $100,000 from the Water Recreation Account, designated as 'other' in this work program, towards this project over a two year period. This support in conjunction with funding that we hope the legislature will appropriate at the recommendation of the LCMR will provide $190,000 for this research. This project will be directed by Luke Skinner with assistance from Chip Welling and Wendy Crowell, both of the DNR.

A. Eurasian watermilfoil
Cooperators at the University of Minnesota include: Drs. Raymond Newman, David Ragsdale, and David Biesboer. Technical expertise on milfoil will be provided by the Army Corps of Engineers.

Cooperator	Dollars received	Percent time spent on project
R. Newman*	$95,000	20%

B. Purple loosestrife
Cooperators at the University of Minnesota include: Drs. Roger Becker, David Ragsdale, and Elizabeth Stamm Katovich. Technical expertise on loosestrife will be provided by Dr. Bernd Blossey of Cornell University, and Dr. Dharma Sreenivasam, Minnesota Department of Agriculture

Cooperators	Dollars received	Percent time spent on project
R. Becker and D. Ragsdale*	$95,000	15% each

*Includes DNR Funding contribution

D. Time: This project is expected to be completed within the time allotted under this work program.

VII. DISSEMINATION: It is expected that the results of this project will be published in peer-reviewed scientific journals and also in special publications and newsletters. Results also will be presented at national, regional and state scientific meetings to peers in the field, as well as to resource managers and planners who will use the results of this project.

<u>In preparation for future submission to peer-reviewed scientific Journals:</u>

McCornack, B. P., L.C. Skinner and D.W. Ragsdale. *In prep*. Landscape-Scale and Within Wetland Movement of *Galerucella* spp. Introduced for Management of Purple Loosestrife (*Lythrum salicaria* L.). For Submission to Environmental Entomology.

Skinner, L.C., E.J. Stamm Katovich, D.W. Ragsdale, W.J. Crowell, N. Proulx and R.L. Becker. *In prep*. Population Dynamics and Long-term Effects of *Galerucella* spp. on Purple loosestrife, *Lythrum salicaria,* and non-target native plant communities in Minnesota. For submission to Biological Control.

Solarz, S.L., R.M. Newman, D.L. Byers, and R.G. Shaw. *In prep*. Heritability, environmental effects and genetic correlations of oviposition preference and fitness components for the milfoil weevil reared on two hosts. For submission to Evolution.

Stamm Katovich, E. J., R.L. Becker, D.W. Ragsdale and L.C. Skinner. *In prep*. Growth and phenology of three Lythraceae species in relation to *Galerucella* spp. For submission to Weed Science.

Ward, D.M. and R.M. Newman. *In prep*. Fish predation on Eurasian watermilfoil herbivores and indirect effects on macrophytes. For submission to Canadian Journal of Fisheries and Aquatic Sciences.

VIII. LOCATION*:* Milfoil research will take place on 7 county Metro area lakes. Loosestrife research site will take place in the 7 county metro area and along the Mississippi river corridor between Red Wing and Winona MN. Site selection for both projects is not complete.

IX. REPORTING REQUIREMENTS: Periodic workprogram progress reports will be submitted not later than January 2002, July 2002, January 2003 July 2003. A final workprogram report and associated products will be submitted by June 30, 2004, or by the completion date as set in the appropriation.

X. RESEARCH PROJECTS: Refer to the attached research proposals for project details (attachment B1 and B2.

ATTACHMENT A

Date: August 18, 2004

Project Title: Biological Control of Eurasian Watermilfoil
and Purple Loosestrife-Continuation

Project Number: 4(D)

LCMR Recommended Funding: $90,000

Attachment A Deliverable Products and Related Budget

2001 LCMR Project Biennial Budget				Objective/ Result											
	Result 1 Budget:	Result 1 Current invoice:	Result 1 Balance:	Result 2 Activity 1 Budget:	Result 2 Current Invoice:	Result 2 Activity 1 Balance:	Result 2 Activity 2 Budget:	Result 2 Current Invoice:	Result 2 Activity 2 Balance:	Result 3:	Result 3 Current Invoice:	Result 3 Balance:	PROJECT TOTAL:		
Budget Item (Title of Result)	Milfoil study			Loosestrife Study			Loosestrife study			loosetrife Study			BUDGET TOTAL:	CURRENT INVOICE TOTAL:	BALANCE TOTAL:
Wages, salaries & benefits – Be specific on who is paid $															
Contracts	45,000		0	15,000		0	10,000		0	20,000		0	90,000		0
Professional/technical (with whom?)	University of MN			University of MN			University of MN			University of MN					
Other contracts (with whom?)															
Space rental: NOT ALLOWED	X			X			X			X			X		
Maintenance															
Utilities															
Other direct operating costs (for what? – be specific)															
Printing															
Advertising															
Communications, telephone, mail, etc.															
Office Supplies (list specific categories)															
Other Supplies (list specific categories)															
Local automobile mileage paid															
Other travel expenses in Minnesota															
Travel outside Minnesota (where?)															
Tools and equipment (list categories)															
Office equipment & computers (be specific)															
Other Capital equipment (list specific items)															
Land acquisition															
Land rights acquisition (less than fee)															
Buildings (for what?)															
Other land improvement (for what?)															
Legal fees (for what?)															
COLUMN TOTAL	45,000	$	0	15,000	$	0	10,000	$	0	20,000	$	0	90,000		0

Biological Control of Eurasian Watermilfoil

Completion Report for 2001-2004

BY

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TO

Charles H. Welling
Eurasian Watermilfoil Program
Ecological Services Section, Box 25
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500 Lafayette Rd.
St. Paul, MN 55155-4025

June 2004

Deliverable A-6. Report of results from 2003 and preceding years.

Content: Processing and analysis of 2003 samples will be completed and the results will be summarized in a multi-page progress report that will be submitted to the MnDNR. Results from all data collected will be analyzed and interpreted. In addition, analysis and synthesis of results from research done in the preceding years over five biennia will be presented.

Introduction

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is an exotic aquatic weed that often interferes with recreation (Smith and Barko 1990), inhibits water flow, impedes navigation, (Grace and Wetzel 1978) and will displace other aquatic macrophytes (Madsen et al. 1991). It was first reported in Minnesota in 1987 and occurred in over 150 Minnesota waterbodies by fall 2003 (Exotic Species Program 2004).

Recent work on the biological control of Eurasian watermilfoil has focused on the indigenous weevil *Euhrychiopsis lecontei* (Dietz) (= *Eubrychiopsis lecontei*), although the caterpillar *Acentria nivea* and the midge *Cricotopus myriophylli* are also potential control agents (Newman 2004). This work suggests that *E. lecontei* is the most promising control agent (Creed and Sheldon 1995, Sheldon and Creed 1995, Creed 1998, Newman and Biesboer 2000). The weevil is native to Minnesota and Wisconsin (Newman and Maher 1995, Jester et al. 1997) and is highly specific to watermilfoils (Solarz and Newman 2001). Sheldon and O'Bryan (1996), Newman et al. (1996, 1997), Mazzei et al. (1999) and Newman et al. (2001) describe the life history and development times of the weevil. Newman (2004) provides a comprehensive review of agents and the biological control of Eurasian watermilfoil.

Although declines of milfoil in several lakes have been related to the occurrence of *E. lecontei* (Sheldon and Creed 1995, Lillie 2000, Newman and Biesboer 2000, Creed 1998), it is clear that at many sites in Minnesota, weevil densities do not get high enough to effect control (Newman et al. 1996, Newman et al. 1998, Newman and Biesboer 2000). Fish predation may be one factor limiting populations in some lakes (Sutter and Newman 1997, Newman and Biesboer 2000, Ward 2002, Newman 2004). Identification and amelioration of factors limiting the milfoil weevil is essential for operational biological control of Eurasian watermilfoil (Newman et al. 1998). Getsinger et al. (2002) provide a good overview of the potential use of the weevil for control of milfoil and Newman (2004) provides a review of limiting factors and success across the country.

The aim of this project is to attempt to detect milfoil declines and assess milfoil weevil populations, identify and manipulate factors that may be limiting control agent densities and identify and manipulate factors that may limit the effectiveness of milfoil control agents (plant community response). This report presents our results from 2001-2003 and summarizes our overall results during the past 10 years and provides some final conclusions and recommendations.

Acknowledgements

Numerous people assisted with this project, including: Chris Lemmon, Darren Ward, Shannon Bishop, Aaron Berger, Kerry Accola, Matthew Dowgwillo, Emily Fisher, Jon German, Jen German, Jordan Greenwood, Heather Hendrixson, Brian Huser, Ruth Isakson, Todd Kittle, Chris Kolasinski, Luke Kramer, Nick Lehnertz, Seth Lengkeek, Jack Lund, William Tanberg and Kim Whorrall. A large number of undergraduates assisted with the project in previous biennia, but the following graduate students and technicians were particularly instrumental: Lynn Mizner, Susan Solarz, Kerry Holmberg, Barb Penner, Mary Kay Corazalla, John Foley, Aaron Berger, Kristine Mazzei, Tom Sutter and Ray Valley.

Methods

Semi-permanent Transect Sites:

During the summers of 1993 and 1994, we initiated selection of semi-permanent sampling sites, which can be repeatedly sampled at fixed locations (Newman and Ragsdale 1995). The sites were Lake Auburn (Carver Co.; T116N; R24W; S10), Otter Lake (Anoka and Ramsey Co.; T30-31N; R22W; S3-4, S35-36), Cedar Lake (Hennepin Co.; T29N; R24W; S29) and Smith's Bay of Lake Minnetonka (Hennepin Co.; T117N; R23W; S10,11). At each site, 5 transects, 30 m apart, were run from near shore (0.5 m depth) toward the plant limit. At Lake Auburn and Cedar Lake, the transects extended to 50 m from the shoreward starting point, in approximately 2.5 m depth at Auburn and 5 m depth in Cedar. Semipermanent stations were marked along the transect at 10 m intervals with fluorescent floats that were attached to bricks and suspended 0.5-1m beneath the surface. At Otter Lake, the transects were extended 100 m from shore, in approximately 2 m depth. At Smith's Bay, transects were started 100 m from shore (1.5m depth) and run to 4.5 m depth, approximately 0.8 km from shore, with 5 sampling stations along each transect approximately geometrically spaced. Distances from shore determined from GPS data were: 100m, 200m, 370m, 585m and 805m. These stations were marked with floating milfoil buoys.

In summer 1996, we noticed a dense population of weevils at Cenaiko Lake (Anoka Co.; T31N; R24W; S26). We therefore sampled this lake in July and September as a new site to be regularly sampled. We ran 3 or 4 transects, west to east across the north end of the lake, with sampling stations every 30 m. This resulted in 25-32 samples on each date (21-30 with plants; deep stations were deleted from the analysis). At Lake Auburn transects were sampled at 10 m intervals (stations) , resulting in 6 samples per transect, or 30 samples. At Otter Lake samples were taken at each 20m sampling station, resulting in 5-6 samples per transect or 27 samples. At Cedar (30) and Smiths Bay (25), all stations were sampled, however, several stations in Cedar Lake were deeper than the plant limit (>7m) and these are excluded if no plants occurred there during the season. In 1997 sampling occurred twice: in late June to early July and in mid-September. In 1998, three lakes (Auburn, Cenaiko and Smith's Bay) were sampled thrice, in June, late-July or early August and in September. Otter and Cedar were sampled in June and September. Samples were alternately taken 2m from each side of each station on successive sampling dates to minimize sampling disturbance. In 1999, two lakes (Cenaiko, and Smith's Bay) were sampled thrice, in June, late-July or early August and in late August. Auburn and Cedar were sampled in June and late August and Otter was sampled in June and early August. In 2000, four lakes were sampled three times (Auburn, Cenaiko, Otter and Smith's Bay), in June, July and August and Cedar Lake was sampled twice, in June and August. Twenty-four to thirty samples were collected at each lake on each date. In 2001, four lakes (Auburn, Cenaiko, Otter and Smith's Bay) were sampled three times, in June, late July and late August. Cedar was sampled in June and August. In 2002 all 5 lakes were sampled twice, in early (late June or early July) and late (late August or early September) summer. In 2003 4 lakes (Auburn, Cedar, Cenaiko, and Otter) were sampled once, in August or early September. Smith's Bay was not sampled in 2003. Twenty to thirty samples were collected at each lake on each date.

At each sampling station, plant biomass and invertebrate samples were taken from 0.1 m^2 quadrats (all plant material was clipped at sediment interface and immediately placed in a sealable bag underwater). Sediment cores were also collected at shallow, medium and deep stations along 3 transects at each site.

A set of water column parameters was measured in the open water (>5.5m depth and >100 m from the bed) at each site on each sampling date. Secchi depth and surface conductivity were measured and a water sample (combined surface and Secchi depth sample) was collected for pH, alkalinity and chlorophyll a determination. A light (Photosynthetically Active Radiation = PAR, Li-Cor LI-189 with LI-192SA quantum sensor), temperature and oxygen (YSI 50B) profile was taken at 0.5 m depth increments from surface to bottom.

Alkalinity was determined by titration. For chlorophyll, 500 ml of water were filtered through a 1.2 mm glass fiber filter, the filter was placed on dry ice and returned to the laboratory and frozen until analysis. Chlorophyll was extracted with buffered acetone and measured spectrophotometrically (APHA 1989). Sediment cores were stored on ice and returned to the laboratory. Within 48 hr the top 15 cm of sediment was homogenized. A 5 ml sediment subsample was dried at 105 °C for 24-48 hrs and then weighed to obtain bulk density (g dry mass ml^{-1}). The dried sediment was then ashed at 550 °C for 4 hrs to obtain percent organic matter ([AFDM dry $mass^{-1}$] X 100). Pore water was extracted from the remaining sediment by centrifugation, acidified to < pH 2 and stored in the refrigerator. The remaining spun sediment was either processed immediately or was frozen for later analysis. In 2001-2003 we further extracted the spun sediment with 2M KCl (shaken for 1 hr) to determine exchangeable nitrogen. The extract was filtered and acidified. Within seven days, the NH_3 concentration was determined for both pore water and KCl-extracted fractions by selective electrode (APHA, 1989). These results should allow us to evaluate McComas's (1999) hypothesis that nuisance levels of milfoil should only appear in sediments with high total nitrogen (e.g., > 3 mgN/L), whereas native plants should dominate in lower nitrogen sediments.

Biomass samples were rinsed of invertebrates and invertebrates were picked (endophytic and external on milfoil and from the wash water) from all samples; weevils and Lepidoptera were enumerated. Milfoil stems were counted and the average maximum stem length determined. Plants were separated, identified to species, spun for 15 sec in a salad spinner and wet mass was recorded. These samples were dried (105 °C for 48h) and weighed or were frozen for later dry mass determination.

Because the relatively infrequent sampling of these sites (2 or 3 times per summer) does not provide very good resolution of weevil population dynamics, we initiated a biweekly weevil survey in Lake Auburn 1998 and in 1999 added Cenaiko and Smiths Bay to our weevil surveys. In 2000 we added Otter to our survey sites and we conducted bi-weekly surveys at Auburn, Cenaiko, Otter and Smith's Bay each year from 2000-2003. For each survey, 5-8 stems (top 50 cm) of milfoil were collected at each of 15-18 stations every other week (at Cenaiko and Otter after declines we were unable to find milfoil at some stations). At sites with lower densities of weevils we have been collected 7 or 8 stems to increase our power to detect weevils. Weevils and Lepidoptera were removed from the samples, which were scanned at 8X magnification, and enumerated by life stage. Results were expressed as numbers per basal stem. Single weevil surveys were also conducted during 2002 in Bald Eagle (Ramsey Co.), Calhoun, Cedar, Centerville (Anoka Co.), Independence (Hennepin Co.), Peltier (Anoka Co.), Schultz (Dakota Co.) and Vadnais (Ramsey Co.) to correlate weevil density with fish density (see below). These surveys were repeated in 2003 at Calhoun and Cedar.

Survey Sites:

In 2001 and previous years, we conducted broader scale (whole lake or bay) surveys of plants in August at 5 sites: Lake Calhoun Hennepin Co.; T28-29N; R24W; S4,5,32,33), Lake Harriet (Hennepin Co.; T28N; R24W; S8,9,16,17), Lake of the Isles (Hennepin Co.; T29N; R24W; S32,33) and Shady Island (Hennepin Co.; T117N; R23W; S26) and Grays Bay (Hennepin Co.; T117N; R22W; S8) in Lake Minnetonka. In 2002 we sampled Calhoun, Cedar, Harriet and Isles, plus Centerville, Schultz and Vadnais. Weevil surveys were conducted on all of the lakes (except Isles, which had little milfoil by August) in 2002 to relate weevil density to sunfish abundance (see below). At each lake, plant community structure was determined with plant hook surveys along 5-15 transects and water quality was recorded. In 2003 we surveyed Calhoun, Cedar, Harriet and Isles.

To quantitatively determine the extent of milfoil coverage, a set of 5-15 transects, perpendicular to shore, was located around the lake or bay in a stratified random manner (i.e., 1 transect located within each 1/10 of the lake shoreline circumference) in August. Along each transect, observations were made from shore (0.5 m depth) to the plant limit at 5 to 6 stations, at 7.5, 15, 30, 60, or 90m intervals to the depth of the plant limit. At steeper transects the shorter intervals were used, at long and gently sloping transects, the longer intervals were used. Transects were laid with a measuring rope and marked with jugs attached to bricks; the shoreward and offshore positions were recorded with a GPS unit. At each observation point, visible milfoil (% coverage) and other plant occurrence was recorded, plant height determined and plant disk (depth at which a Secchi disk disappears; Crowell et al. 1994) was measured within a 1m^2 area around the marker jug. Depth was recorded by dropping a plant hook vertically; plant species found on the plant hook or the jug rope and brick were also recorded and milfoil was examined for weevils and given a weevil damage rating (0-5). These data provide an estimate of milfoil and other plant coverage and frequency of occurrence around the lake as well as a relative estimate of weevil damage or occurrence.

Semi-quantitative estimates of plant density and weevil abundance were determined along a stratified subset of 5 of the transects with modification of a grapple hook method of Jessen and Lound (1962). At each sampling point 3 or 4 grapple throws were collected and rated for plant occurrence and density on a scale of 0-5 (Jessen and Lound 1962); these data provide species occurrence and relative density estimates for each species. The milfoil collected on each throw was scanned for the presence of weevils and visually assigned a damage rating (0-5). Thus for these 5 transects, we have both visual estimates of plant occurrence and density as well as the semiquantitative plant hook estimates.

Localized sites at Calhoun, Harriet and Isles were sampled quantitatively for milfoil, invertebrates and site characteristics in 2001-2003. At Calhoun, Lake of the Isles and Harriet, 5 transects with 5 stations on each transect were sampled twice in 2001 (June and August) and once in 2002 and 2003 (August). At each station 0.1m^2 quadrat samples were taken for plants and invertebrates. Sediment cores were sampled at the intermediate depth station along each transect. Open-water water quality samples were taken and processed in the same manner as the permanent transect sites. Samples were processed as above for plant mass by species, weevil abundance, and sediment characteristics.

Relationship of Weevil and Sunfish Densities:

Because previous research suggested that high sunfish densities were limiting weevil populations, we selected a set of lakes for which recent DNR fish population assessment were conducted and conducted single weevil surveys in late July or August 2002. These lakes were Bald Eagle (Ramsey Co.), Calhoun, Cedar, Centerville (Anoka Co.), Independence (Hennepin Co.), Peltier (Anoka Co.), Schultz (Dakota Co.) and Vadnais (Ramsey Co.). At each lake, 5 transects were established around the lake and 4 stations (from shore to deep edge of the bed) on each transect were sampled for herbivores by collecting 8 milfoil stems (top 50 cm). These plants were processed and herbivores enumerated as done for other weevil surveys. At five of these lakes we also conducted plant community surveys (see above) to see if declines in milfoil were related to weevil or sunfish density.

The DNR fisheries survey results for trapnet catches of all sunfish (bluegill, pumpkinseed, bluegill X pumpkinseed hybrids and green sunfish) were used to estimate relative sunfish density (mean catch per overnight trapnet set). Most fisheries assessments were conducted in 2002, but assessments on Independence and Vadnais were conducted in 2001 and Calhoun and Cedar in 2000. Regression of our single-sample summer weevil density estimates with sunfish abundance was used to determine if there is an among-lake relationship of weevil density with sunfish density. To increase sample size, we also obtained DNR fisheries population assessments for the lakes on which we have been conducting regular bi-weekly weevil surveys. Fisheries assessments were available for Auburn in 2000, Cenaiko in 1998 and 2002 and Otter in 2001 and 2002. For these lakes we used average summer weevil densities for the year in which the fisheries assessment was conducted.

Weevil Introduction/Manipulation:

Previously we conducted small-scale augmentations in caged fish exclosures and enclosures (Ward 2002). To provide a more realistic assessment of the feasibility of stocking or augmenting weevil populations we stocked weevils into two lakes with low weevil populations and different sunfish densities in 2002: Harriet and Hiawatha. Based on prior DNR fisheries assessments, Harriet was considered a high sunfish lake (340/trapnet) and Hiawatha a low sunfish lake (11/trapnet). An herbivore (weevil) stem survey (5 transects, 4 stations) was conducted prior to stocking to determine weevil abundance (no weevils were found in these surveys).

In mid-July, two contiguous plots (approximately 120m along shoreline to the deep edge of milfoil bed, each plot was ≥ 100m apart) were chosen in each lake and plant biomass and herbivore densities were determined with quantitative 0.1 m^2 quadrat samples from 4 stations (shallow to deep) on three transects in each plot (12 samples per plot). Adult weevils and associated meristems (including eggs and larvae) were collected from Otter Lake and 3000 adult weevils were stocked into one randomly selected plot in each lake in mid-July 2002. Meristems (with adults and associated eggs and larvae) were tied to individual plants with biodegradable twine. Biweekly weevil (herbivore) stem surveys (12 stations per plot, 8 stems per station) were conducted to monitor weevil populations and in mid-September 2002, 12 quadrat samples were collected from each plot to determine plant biomass and areal herbivore densities. The lakes were re-sampled for biomass in June of 2003 and biweekly weevil surveys were conducted through summer 2003. In July 2003 an additional 2000 adult weevils were stocked into each lake and biomass was again sampled at the end of the summer.

Effects of plant community:

To test the hypothesis that plant competition may be important in the reestablishment of Eurasian watermilfoil after a decline (or reduction due to weevil damage) we established plots in Otter Lake (good water clarity and healthy native plant community) and in Lake Auburn (poor water clarity with community dominated by Eurasian watermilfoil and coontail) for plant community manipulation experiments. Initial experiments were conducted in 1998-1999.

We established a new set of plant manipulation plots in Otter Lake and Lake Auburn in 2001 and in Cedar (good clarity but low diversity) in 2002. At each lake we established 20 plots marked by 2mx2m pvc quadrats. The plots were sampled in early June for plant biomass (2 0.1-m^2 quadrat samples per plot) prior to manipulation. After initial sampling, the randomly assigned manipulation was applied to the plot by divers using SCUBA who manually removed vegetation within the area delineated by the 2x2 PVC quadrat. Harvested vegetation was not retained but allowed to float away. In five plots no plants were removed, in 5 plots all plants were removed and in the other plots either all native plants or all Eurasian watermilfoil was removed. Several times each summer, visual surveys (means of 16 0.5x0.5 cells) of plant coverage were conducted and in September, two biomass samples were taken from each plot. Otter Lake and Lake Auburn were re-sampled for biomass in June and September 2002 and visual surveys were conducted several times during summer 2002 to further follow community changes. In 2003, the removal plots in Cedar and Otter were resampled for biomass in late June or early July. The duplicate biomass samples within plots were averaged and statistical analyses were conducted on the replicate plots. We collected sediment cores from each plot in Otter Lake in September 2001 and 2002 and June 2003 and from each plot in Cedar and Auburn in September 2002 and Cedar in July 2003.

Relationship of plant community to sediment characteristics:

McComas (1999) proposed that sediment nitrogen may be a good predictor of nuisance levels of Eurasian watermilfoil; high nitrogen sites should support dense growths of milfoil while lower nitrogen sites would be more amenable to native plants that are adapted to lower nitrogen levels. At low nitrogen sites, Eurasian watermilfoil should not reach nuisance levels. Recently, McComas (2003) updated his predictions and predicted that nuisance milfoil should occur in sediments with > 6ppm exchangeable ammonia. This prediction was based on a volume basis (mg/cm^3, McComas, personal communication). In 2001 we started measuring exchangeable (KCl extractable ammonium) N from the sediments because pore water ammonium is rapidly influenced by short-term plant uptake and may not reflect longer-term nitrogen availability. We analyzed all the sediment samples from 2001-2003 for exchangeable N (see above for methods). We report exchangeable N from the KCl extract as well as total exchangeable N (KCl extract plus pore water nitrogen). Although our measures based on dry mass (mg N/g dm sediment) are not directly comparable to McComas's, they should provide some basis for testing his hypothesis and an assessment of possible N limitation of milfoil at our sites.

Results and Discussion

Semi-permanent Transect Sites:

Milfoil and total plant biomass fluctuated over time and differed among lakes (Fig. 1); annual climatic factors do not appear to be the main determinants of milfoil biomass at these sites.

Lake Auburn showed large changes in milfoil biomass over time, increasing to high levels in 1995-1996, followed by a decline from 1998-2000 with a slow increase from 2001-2002 and another decline in 2003 (Table 1). Plants other than milfoil also increased in 1995 and generally remained over 1000 g wet/m^2 through 2001 (Table 2). Non-milfoil biomass dipped in 2002, but returned to near 1000 g/m^2 in 2003. During years of high milfoil biomass, milfoil composed 60-90% of total plant biomass, but during 1998-1999 it composed <40% of total plant biomass (Table 3). Biomass of non-milfoil plants at Auburn was dominated by coontail (Fig. 1) and generally only 2-3 species were found per sample (Table 2). The total number of species found per date ranged from 3 to 12 (Table 3) with 6-9 species being typical. Milfoil biomass was not significantly correlated with coontail or other plants and the plant community varied independently.

Lake Auburn had fertile sediments with an intermediate bulk density (0.4-0.6 g dm/ml) and percent organic matter (10-20%; Table 4). Pore water ammonium tended to be suppressed with high densities of plants. Water clarity was fair to poor at Lake Auburn; late summer Secchi depths were less than 2m in about half the years, but low Secchi depths in 1997 and 2001 did not appear to suppress milfoil growth, so it is unclear if equally poor clarity in 1998 and 1999 was responsible for the low biomass in those years. Changes associated with herbivores are addressed in the following section.

Cedar Lake showed less variation in milfoil and total plant biomass. Biomass was low in 1996, despite fair water clarity (Table 4), and increased to more than 2500 g/m^2 in 1997 and 1998 following alum treatments (and improved clarity) before returning to slightly lower levels between 1500 and 2000 g/m^2. Biomass of non-milfoil plants was typically < 1000 g/m^2 (Table 2) and was dominated by coontail. Cedar consistently had the lowest mean number of species per sample among the lakes, typically < 2 species per sample (milfoil and coontail). It also had the lowest total number of species; occasionally 5 species were found but 2-4 species were more typical (Table 3). As with Auburn, milfoil biomass was not significantly correlated with coontail or other plants. Cedar Lake sediments were similar to Auburn with an intermediate bulk density and percent organics (Table 4). Poor late summer clarity in 1995 may have suppressed milfoil and the improved clarity after alum treatment in 1996 appeared to enhance milfoil biomass in 1997-1999.

Otter Lake had a high biomass of milfoil in 1994 and 1995 (Table 1), when it composed 75-95% of total plant biomass (Table 3). A dramatic decline in milfoil biomass occurred over the winter of 1995-1996; milfoil biomass was extremely low in June 1996 and dropped to zero by the end of the summer. This decline was likely due to a severe winterkill that killed the stems, root crowns and roots of the milfoil plants. Native plants, many which reproduce from seed, increased over the summer and remained dominant through 1999 (Table 3). Milfoil slowly increased and reached a peak of 2600 g/m^2 in June 2000 and then declined with increasing herbivore densities (see below). Milfoil remained at <30% of biomass until 2003 when it increased to 40% (Table 3).

In contrast to Auburn and Cedar, Otter Lake had a higher diversity of native plants; typically 9-15 species were found (3-5 species per sample) and even during years of high milfoil biomass, 9-12 species were found. Milfoil was not significantly correlated with coontail, but it was negatively correlated with other plants ($r= -0.46$, $p<0.05$) and coontail was marginally negatively correlated with other plants ($r= -0.38$. $p<0.1$). When milfoil was suppressed, rooted native plants colonized and coontail did not become dominant. Otter Lake sediments had a lower bulk density and higher organic content than the other lakes (Table 4) and better Secchi depths than Auburn (typically >2m throughout the summer).

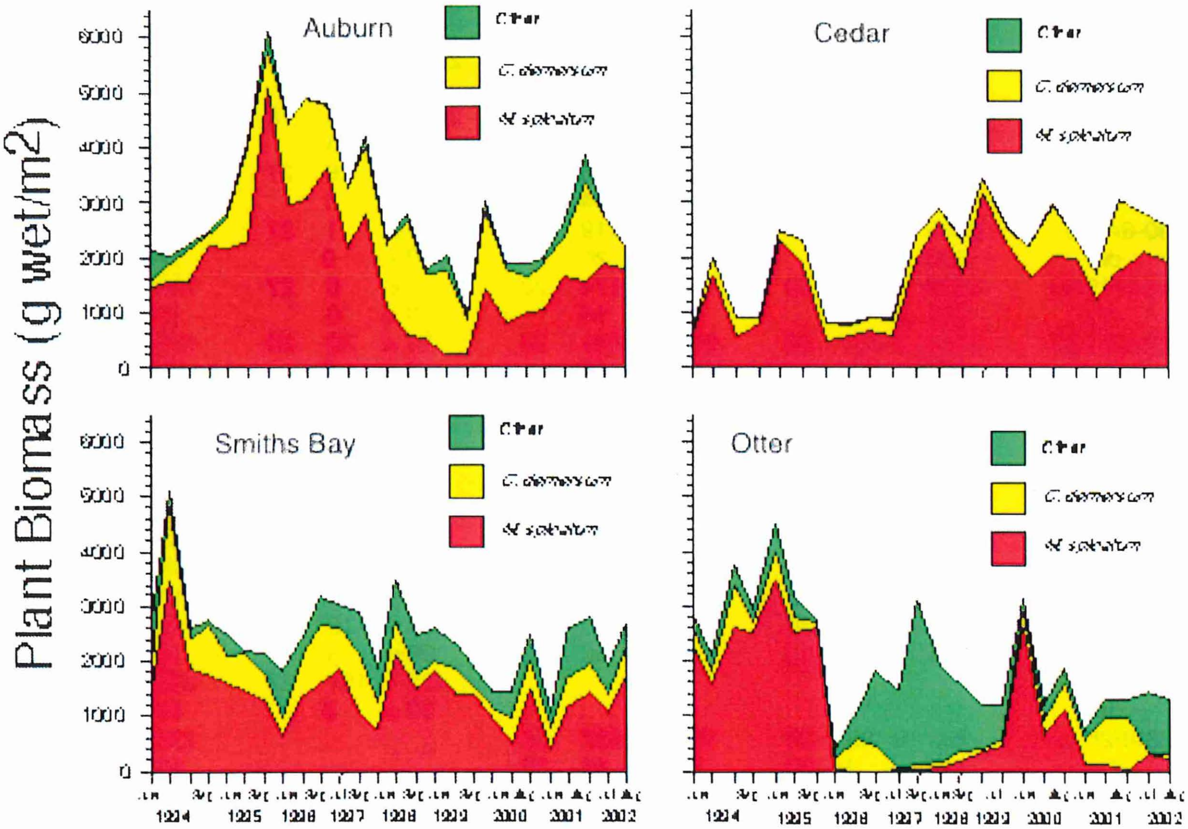

Fig. 1. Total plant biomass (Eurasian watermilfoil, coontail and other non-milfoil biomass; g wet/m^2) at the four permanent transect sites from May 1994 - August 2002.

Smith's Bay generally had the most consistent milfoil density. After a peak biomass of 3500 g/m^2 in 1994, milfoil only exceeded 2000 g/m^2 once (1998) and typically ranged from 800-1500 g/m^2 (Table 1) and composed 40-60% of total plant biomass. Like Otter, the plant community was more diverse and 10-15 species were commonly found with a mean of 3-4 species per sample. Non-milfoil biomass ranged from 600-1800 g/m^2 and coontail typically composed 20-50% of non-milfoil biomass. At Smith's Bay, milfoil and coontail biomass were significantly positively correlated ($r=0.58$. $p<0.01$) but neither milfoil nor coontail were correlated with other plant density. Smith's Bay had the best water clarity of the sites and Secchi depths typically exceeded 2.5m throughout the summer (Table 4). Sediment bulk density was slightly lower than Cedar but percent organics were also lower, generally ranging from 10-15%.

Table 1. Biomass ± 1SE (g wet/m^2) of Eurasian watermilfoil at the four sampling sites in 1994-2003. n = number of samples. Dry biomass (g/m^2 ± 1SE) is presented for 1995-2003.

Sampling Date	Auburn	n	Cedar	n	Otter	n	Smith's Bay	n
5/19-6/3/94	1474 ± 326	10	610 ± 289	18	2208 ± 332	21	1470 ± 320	14
7/1-7/11/94	1570 ± 297	16	1642 ± 523	18	1589 ± 231	27	3478 ± 399	16
8/12-8/19/94	1581 ± 224	15	601 ± 207	15	2626 ± 472	14	1886 ± 328	16
9/14-9/21/94	2205 ± 350	19	824 ± 188	24	2510 ± 557	9	1767 ± 386	14
6/07-6/27/95	1999 ± 324	30	2307 ± 631	23	3444 ± 336	27	1618 ± 289	25
dry	280 ± 43		245 ± 67		312 ± 33		158 ± 28	
7/31-8/15/95	2277 ± 417	19	1821 ± 797	10	2526 ± 385	15	1481 ± 245	25
dry	267 ± 46		172 ± 79		171 ± 29		149 ± 28	
9/18-9/29/95	5044 ± 752	17	479 ± 173	17	2629 ± 323	18	1281 ± 178	25
dry	551 ± 94		37 ± 13		194 ± 23		113 ± 15	
6/12-6/24/96	2959 ± 402	30	568 ± 200	30	21± 8	27	665 ± 144	25
dry	306 ± 40		59 ± 24		2 ± 1		46 ± 10	
7/30-8/9/96	3035 ± 619	27	665 ± 219	30	1± 1	27	1415 ± 256	25
dry	390 ± 82		62 ± 20		0 ± 0		176 ± 36	
9/12-9/19/96	3622 ± 469	30	574 ± 174	30	0± 0	27	1656 ± 393	25
dry	361 ± 49		50 ± 14		0 ± 0		156 ± 40	
6/27-7/17/97	2134 ± 321	30	1906 ± 341	28	24 ± 22	26	1880 ± 327	25
dry	294 ± 46		210 ± 40		3 ± 3		296 ± 55	
9/8-9/18/97	2786 ± 400	30	2646 ± 502	29	4 ± 4	27	1055 ± 170	25
dry	321 ± 49		271 ± 55		0 ± 0		100 ± 18	
6/8-6/18/98	1080 ± 168	30	1690 ± 360	31	79 ± 52	27	815 ± 164	25
dry	130 ± 18	30	213 ± 52	31	7 ± 4	27	105 ± 21	25
7/27-8/3/98	581 ± 133	30					2103 ± 475	25
dry	67 ± 16	30					286 ± 65	25
9/8-9/16/98	530 ± 76	30	3146 ± 514	29	181 ± 44	27	1487 ± 338	25
dry	48 ± 7	30	367 ± 63	29	15 ± 4	27	172 ± 40	25
6/15-6/22/99	202 ± 50	30	2238 ± 393	28	355 ± 113	27	1806 ± 289	25
dry	24 ± 7	30	252 ± 50	28	25± 8	27	155 ± 32	25
7/29-8/3/99					483 ± 101	27	1358 ± 289	25
dry					36 ± 8	27	189 ± 44	25
8/23-8/25/99	253 ± 83	30	1632 ± 237	30			1362 ± 320	25
dry	25 ± 9	30	105 ± 15	30			106 ± 26	25
6/6-6/23/00	1392 ± 263	30	2045 ± 321	29	2652 ± 340	27	981 ± 318	25
dry	208 ± 39	30	219 ± 38	29	331 ± 42	27	109 ± 37	25
7/11-7/19/00	783 ± 200	30			607 ± 82	27	501 ± 150	25
dry	115 ± 32	30			45 ± 7	27	77 ± 22	25
8/23-8/29/00	1007 ± 152	30	1988 ± 305	29	1098 ± 136	27	1474 ± 346	25
dry	91 ± 14	30	175 ± 28	29	90 ± 14	27	162 ± 40	25
6/18-6/25/01	1022 ± 199	30	1213 ± 267	29	116 ± 34	27	408 ± 107	25
dry	109 ± 21	30	111 ± 26	29	9 ± 3	27	31 ± 8	25
7/17/-7/30/01	1641 ± 279	30			138 ± 58	25	1211 ± 290	25
dry	232 ± 45	30			6 ± 3	27	168 ± 43	25
8/23-8/30/01	1549 ± 289	30	1798 ± 398	25	24 ± 11	27	1438 ± 381	25
dry	158 ± 33	30	162 ± 41	25	2 ± 1	27	160 ± 43	25
6/2-7/8/02	1886 ± 339	30	2123 ± 468	21	302 ± 87	30	1067 ± 245	25
dry	254 ± 46	30	231 ± 52	21	28 ± 7	30	137 ± 36	25
8/8-9/6/02	1776 ± 273	30	1910 ± 294	32	205 ± 49	30	1746 ± 346	25
dry	222 ± 37	30	149 ± 23	32	13 ± 3	30	246 ± 47	25
8/8/-9/19/03	346 ± 98	25	1564 ± 338	25	1073 ± 241	18		
dry	22 ± 6	25	132 ± 32	25	74 ± 20	18		

Table 2. Mean number of species per sample (Spp/S) ± 1SE and non-milfoil biomass (B; g wet /m^2) at the 4 sampling sites in 1994-2003. Number of samples is given in Table 1.

Sampling Date	Auburn		Cedar		Otter		Smith's Bay	
	Spp/S	B	Spp/S	B	Spp/S	B	Spp/S	B
5/19-6/3/94	3.80±0.47	670	1.33±0.28	75	4.76±0.19	600	3.29±0.22	1231
7/1-7/11/94	3.63±0.29	444	1.83±0.28	370	4.37±0.29	520	3.75±0.35	1604
8/12-8/19/94	3.00±0.28	647	1.53±0.26	282	5.57±0.39	1126	3.13±0.42	765
9/14-9/21/94	3.11±0.37	268	1.46±0.19	54	4.89±0.61	431	3.50±0.39	975
6/07-6/27/95	2.23±0.22	822	1.43±0.20	214	4.70±0.21	1065	3.64±0.30	877
7/31-8/15/95	3.37±0.26	1789	1.70±0.15	516	4.27±0.30	642	2.68±0.24	703
9/18-9/29/95	2.18±0.18	1058	1.41±0.17	337	2.44±0.34	135	2.80±0.20	856
6/12-6/24/96	2.93±0.24	1450	2.10±0.22	248	5.19±0.25	434	4.32±0.36	1159
7/30-8/9/96	2.78±0.31	1186	1.43±0.18	270	4.19±0.20	1171	3.88±0.41	1017
9/12-9/19/96	2.50±0.20	1166	1.57±0.16	307	3.93±0.28	1798	3.88±0.32	1531
6/27-7/17/97	2.97±0.14	1435	1.82±0.14	460	4.31±0.29	1516	4.16±0.39	1162
9/8-9/18/97	2.63±0.17	1500	1.59±0.09	235	4.81±0.26	3180	3.64±0.27	1863
6/8-6/18/98	2.43±0.18	1158	1.74±0.81	637	5.37±0.24	1835	5.32±0.43	1038
7/27-8/3/98	2.97±0.23	2197					5.00±0.44	1385
9/8-9/16/98	2.40±0.12	1258	1.62±0.12	296	4.74±0.39	1423	4.32±0.38	969
6/15-6/22/99	3.07±0.16	1806	1.86±0.13	326	4.52±0.31	825	4.60±0.37	810
7/29-8/3/99					5.33±0.30	720	3.72± 0.31	973
8/23-8/25/99	1.93±0.13	679	1.37±0.09	570			2.92± 0.33	534
6/6-6/23/00	3.17±0.19	1597	1.62±0.10	919	4.33±0.28	471	3.44±0.39	458
7/11-7/19/00	2.70±0.20	1090			4.59±0.24	595	4.48±0.45	949
8/23-8/29/00	2.30±0.12	852	1.62±0.10	354	4.33±0.21	778	4.00±0.36	979
6/18-6/25/01	2.77±0.21	971	1.52±0.11	495	4.44±0.23	628	4.00±0.35	663
7/17/-7/30/01	2.40±0.11	996			3.04±0.24	1189	3.96±0.32	1387
8/23-8/30/01	2.80±0.16	2314	1.80±0.08	1303	3.81±0.27	1293	3.60±0.28	1342
6/2-7/8/02	2.17±0.11	861	1.67±0.11	738	3.53±0.26	1128	3.28±0.26	858
8/8-9/6/02	2.30±0.14	398	1.53±0.12	709	4.53±0.25	1094	3.12±0.19	928
8/8/-9/19/03	1.92±0.11	993	1.76±0.13	1596	4.67±0.26	1552		

Table 3. Percentages of total plant wet biomass that was Eurasian watermilfoil (±1SE) and total number of species (N) collected at each site. These are the average percentage found in the samples and are thus not equal to total mean milfoil biomass/plant biomass.

Sampling Date	Auburn	N	Cedar	N	Otter	N	Smith's Bay	N
5/19-6/3/94	65% ±10%	9	67% ±11%	4	80% ± 6%	9	64% ±10%	8
7/1-7/11/94	79% ± 6%	9	67% ± 9%	4	75% ± 5%	9	72% ± 6%	11
8/12-8/19/94	74% ± 6%	9	61% ±13%	3	75% ± 6%	11	81% ± 5%	11
9/14-9/21/94	91% ± 6%	9	87% ± 5%	4	83% ± 6%	11	71% ± 8%	9
6/07-6/27/95	72% ± 7%	7	82% ± 7%	3	79% ± 4%	9	61% ± 5%	10
7/31-8/15/95	58% ± 7%	7	58% ± 6%	2	80% ± 7%	9	63% ± 6%	11
9/18-9/29/95	81% ± 7%	5	38% ± 5%	2	95% ± 1%	6	63% ± 7%	10
6/12-6/24/96	70% ± 7%	7	57% ± 7%	5	7% ± 5%	9	33% ± 6%	10
7/30-8/9/96	56% ± 8%	7	59% ± 9%	5	0.1% ± 0.1%	10	56% ± 7%	11
9/12-9/19/96	69% ± 6%	8	73% ± 6%	4	0% ± 0%	9	49% ± 7%	10
6/27-7/17/97	53% ± 13%	10	82% ± 9%	3	1% ± 2%	12	54% ± 14%	12
9/8-9/18/97	60% ± 13%	8	88% ± 9%	2	0.2% ± 0.3%	13	40% ± 14%	11
6/8-6/18/98	42% ± 5%	11	79% ± 5%	4	4% ± 2%	15	37% ± 6%	15
7/27-8/3/98	24% ± 4%	12					49% ± 8%	16
9/8-9/16/98	34% ± 4%	7	82% ± 6%	4	20% ± 5%	13	50% ± 8%	13
6/15-6/22/99	14% ± 4%	7	82% ± 6%	3	30% ± 6%	13	61% ± 7%	12
7/29-8/3/99					40% ± 5%	14	53% ± 8%	13
8/23-8/25/99	36% ± 7%	6	85% ± 6%	2			61% ± 8%	12
6/6-6/23/00	43% ± 6%	9	75% ± 7%	5	81% ± 5%	12	49% ± 9%	13
7/11-7/19/00	37% ± 6%	9			53% ± 4%	15	40% ± 8%	15
8/23-8/29/00	55% ± 6%	6	77% ± 6%	3	63% ± 5%	9	50% ± 8%	13
6/18-6/25/01	52% ± 6%	10	77% ± 6%	2	20% ± 5%	15	35% ± 8%	14
7/17/-7/30/01	56% ± 6%	5			9% ± 4%	11	42% ± 7%	14
8/23-8/30/01	40% ± 6%	5	59% ± 8%	2	5% ± 3%	12	42% ± 8%	12
6/2-7/8/02	65% ± 6%	6	63% ± 9%	2	26% ± 5%	13	44% ± 8%	11
8/8-9/6/02	76% ± 5%	6	73% ± 7%	4	26% ± 5%	16	52% ± 8%	11
8/29/03	32% ± 7%	3	55% ± 9%	4	39% ± 6%	14		

Table 4. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium and water column characteristics in 1995-2003 at the four permanent transect sites. Sediment samples were collected from shallow, moderate and deep stations along transects 1, 3 and 5 (n=9). Secchi depth (SD), chlorophyll a (Chl-a; pooled surface and SD sample) and light and temperature profiles were taken in deep water > 100 m from the plant bed. Temperature is at 1m depth and 10% PAR depth is the depth at which light intensity was 10% of surface light (presented as the range which encompassed the 10% value).

Lake/Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/m^3)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Plant Limit (m)
Auburn								
6/15/95	0.60	3.96	11.34	9.5	2.3	20.7	2.5-3.0	3.0
2se	0.15	0.91	3.73					
8/1/95	0.49	4.00	10.69	13.9	1.4	26.0	1.5-2.0	3.0
2se	0.18	1.24	4.39					
9/26/95	0.45	4.40	12.67	8.0	2.0	14.8	2.5	3.0
2se	0.13	1.96	4.05					
6/13/96	0.41	3.08	16.0	2.9	4.2	25.1	3	3.0
2se	0.11	1.66	8.6					
7/31/96	0.42	5.81	13.6	12.8	2.4	23.3	1-1.5	3.0
2se	0.17	1.52	4.7					
9/12/96	0.38	2.68	13.7	8.8	2.4	21.2	2.5-3.0	3.0
2se	0.14	0.95	4.3					
6/23/97	0.59	1.93	25.6	11.2	1.2	24.5	2.0	3.4
2se	0.22	0.56	16.8					
9/8/97	0.48	4.42	12.3	16.6	1.4	22.4	1.5-2.0	3.4
2se	0.14	1.46	3.3					
6/8/98	0.23	11.82	11.9	14.4	1.9	18.8	1.5-2.0	
2se	0.08	4.07	4.4					
7/28/98	0.45	20.09	9.5	41.2	0.7	25.7	0.5-1.0	
2se	0.27	3.68	4.3					
9/9/98	0.44	37.72	11.9	36.4	1.1	21.9	1.0-1.5	
2se	0.15	12.57	4.6					
6/22/99	0.50	2.79	13.6	9.4	1.8	22.4	2.0	
2SE	0.16	1.06	3.8					
8/23/99	0.44	10.98	11.6	11.0	1.5	23.1	1.0-1.5	
2SE	0.12	1.81	4.2					
6/19/00	0.51	2.36	11.1	5.9	2.1	20.4	2.5-3.0	
2se	0.14	0.51	4.0					
7/17/00	0.57	4.61	10.2	5.3	2.5	25.3	2.5-3.0	
2se	0.22	1.54	3.6					
8/28/00	0.53	7.75	11.8	5.3	2.3	24.3	3.0	
2se	0.14	1.58	3.9					
6/15/01	0.50	0.98	11.2	6.7	2.9	21.5	3	
2se	0.18	0.38	4.2					
7/17/01	0.57	3.72	25.7	7.2	1.8	27.9	2.5	
2se	0.26	1.92	30.5					
8/29/01	0.47	5.46	10.9	0.8	1.7	24.3	2-2.5	
2se	0.18	1.11	3.8					
6/27/02	0.53	6.61	18.8	-	1.6	26.2	2-2.5	
2se	0.12	3.25	6.3					
9/6/02	0.62	5.14	19.7	17.1	2.6	21.0	2.5	
2se	0.22	-	10.4					
8/29/03	0.35	3.71	11.3	.	1.9	25	2.0	
2se	0.10	1.86	3.5					
Cedar								
6/28/95	0.62	3.90	13.73	10.2	4.5	24.0	4.5	4.0
2se	0.36	1.63	6.00					
8/3/95	0.45	7.27	16.41	16.3	1.2	26.7	1.0-1.5	3.1
2se	0.33	1.39	7.40					
9/28/95	0.43	6.06	21.56	27.5	0.8	14.8	1.0-1.5	3.1
2se	0.36	1.98	7.38					

Table 4 Continued

Cedar								
6/18/96	0.57	3.78	13.3	1.1	5.5	24.6	3.5-4.0	6.5
2se	0.38	1.34	6.3					
8/1/96	0.42	3.86	19.0	4.5	1.9	23.8	2.5-3.0	3.1
2se	0.38	1.59	7.5					
9/16/96	0.41	5.12	18.5	5.3	2.8	20.1	2-2.5	3.1
2se	0.37	1.63	6.9					
7/8/97	0.54	3.97	12.89	9.6	2.5	21.0	3.0-4.0	6.0
2se	0.40	2.87	5.97					
9/11/97	0.42	5.69	15.76	0.8	3.7	22.0	3.0-3.5	6.4
2se	0.33	2.26	6.31					
6/18/98	0.31	4.01	18.35	2.1	4.7	22.6	4.5-5.0	
2se	0.30	1.99	5.27					
7/24/98*	N.A.	N.A.	N.A.	1.3	4.7	26.0	4.5-5.0	
9/16/98	0.29	34.77	18.68	6.9	2.6	23.4	2.5-3.0	
2se	0.30	18.72	4.78					
6/23/99	0.51	4.68	16.15	5.3	2.6	25.6	3.5	
2SE	0.36	1.68	8.79					
8/24/99	0.36	12.35	12.14	17.6	1.6	22.9	2.0-2.5	6.1
2SE	0.34	3.87	3.37					
6/23/00	0.32	2.29	18.28	5.1	3.3	23.1	3.0-3.5	
2se	0.25	1.42	4.77					
8/8/00	0.52	4.15	16.89	4.3	1.6	25.9	3.5-4.0	4.6
2se	0.40	3.91	8.43					
6/19/01	0.60	3.83	22.49	15.0	1.9	22.9	3	
2se	0.43	2.14	16.81					
8/30/01	0.45	2.87	14.92	15.8	1.8	24.7	3-3.5	5.0
2se	0.40	0.74	5.99					
7/8/02	0.51	6.11	30.7	-	1.9	28.3	3.5	
2se	0.28	2.51	11.6					
8/30/02	-	-	-	-	2.2	24.6	2.5-3.0	7.8
2se	-	-	-					
8/5/03	0.23	5.08	26.4	.	1.4	25.3	2.5	5.8
2se	0.14	2.62	14.2					
Otter								
6/26/95	0.42	3.27	20.26	5.6	3.0	30.0	3.5-4.0	4.0
2se	0.18	1.43	7.23					
8/10/95	0.39	4.66	24.44	12.5	2.5	24.7	1.5-2.0	4.0
2se	0.26	1.77	9.49					
9/30/95	0.38	2.76	25.07	3.7	1.1	14.5	1.0-1.5	4.0
2se	0.26	1.34	11.34					
6/20/96	0.47	4.86	23.5	8.5	1.9	21.1	1.5-2.0	3.5
2se	0.34	1.67	10.2					
8/6/96	0.27	3.54	27.5	4.8	2	26	2-2.5	4.0
2se	0.16	0.88	8.6					
9/17/96	0.33	3.77	24.9	8.0	1.5	17.9	1.5-2.0	4.0
2se	0.24	1.76	9.5					
7/2/97	0.33	1.89	26.42	9.9	1.3	21.1	2.0-2.5	3.5
2se	0.21	1.09	8.17					
9/15/97	0.29	5.88	27.47	4.8	2.1	21.0	2.0-2.5	3.5
2se	0.16	2.61	9.52					
6/10/98	0.18	10.51	24.24	2.9	2.6	17.8	4.5-5.0	
2se	0.11	3.55	8.54					
9/10/98	0.24	27.47	24.36	1.6	4.0	21.1	3.5-4.0	
2se	0.11	9.40	7.55					
6/21/99	0.24	3.37	27.31	15.5	2.7	24.5	2.5	
2SE	0.07	0.83	8.34					
7/29/99	0.22	9.58	25.37	13.4	2.1	26.4	2.0	
2SE	0.12	3.02	8.61					
7/11/00	0.47	2.69	21.36	6.9	2.5	26.7	1.5-2.0	
2se	0.32	1.63	9.13					
8/29/00	0.25	3.16	29.84	4.5	2.9	23.7	2.0-2.5	
2se	0.13	1.69	9.13					

Table 4 Continued
Otter continued

6/21/01	0.34	2.55	25.25	3.2	2.9	22.5	2.5	
2se	0.20	1.07	10.83					
7/18/01	0.36	3.64	27.71	3.2	2.1	27.8	2.0-2.5	
2se	0.21	1.38	9.70					
8/28/01	0.35	2.77	23.05	5.1	2	24.9	2.5-3.0	
2se	0.19	1.13	8.12					
6/26/02	0.34	5.86	19.5	-	2.6	24.8	2-2.5	
2se	0.20	4.74	12.1					
9/5/02	0.70	6.92	40.2	6.1	2.3	23.7	2.5-3.0	
2se	0.50	3.31	14.1					
9/18/03	0.15	4.62	32.8		3	20.2	2.5-3.0	
2se	0.06	0.84	6.4					
Smith's								
6/29/95	0.59	5.18	11.81	4.0	3.9	23.7	5.0	5.0
2se	0.25	3.40	4.62					
8/16/95	0.28	4.06	12.86	7.5	2.1	24.9	3.5-4.0	5.0
2se	0.14	0.97	3.71					
9/18/95	0.31	4.25	12.50	10.7	2.1	14.7	2.5	5.0
2se	0.15	0.77	3.98					
6/24/96	0.36	1.13	13.9	3.7	3.7	20.6	3.5-4.0	5.0
2se	0.22	0.32	4.7					
8/8/96	0.37	2.61	17.6	1.3	3.4	24.4	4.5-5.0	5.0
2se	0.21	1.01	5.3					
9/19/96	0.32	2.43	19.1	3.2	3.5	20.1	3.0-3.5	5.0
2se	0.18	0.90	14.3					
7/15/97	0.34	2.44	9.29	1.6	3.5	22.2	4.5-5.0	5.0
2se	0.17	0.80	3.48					
9/18/97	0.31	2.94	14.10	5.3	2.4	20.9	2.5-3.0	5.0
2se	0.17	1.21	4.74					
6/15/98	0.35	3.35	11.50	1.6	3.6	21.0	4.0-4.5	
2se	0.19	1.98	4.22					
8/4/98	0.34	9.32	11.76	4.0	2.9	23.6	3.5-4.0	
2se	0.16	3.27	3.59					
9/15/98	0.30	26.00	13.55	4.3	2.7	22.5	3.0-3.5	
2se	0.14	5.87	3.40					
6/16/99	0.34	2.21	12.71	4.3	3.7	20.8	4.0	
2SE	0.18	0.40	4.08					
8/4/99	0.37	11.54	10.32	4.8	2.6	26.1	4.5-5	
2SE	0.22	8.83	3.84					
8/25/99	0.30	9.71	10.63	7.2	2.9	24.7	4.0	
2SE	0.16	3.24	3.52					
6/20/00	0.39	2.03	11.06	4.3	3.2	19.9	4.0-4.5	
2se	0.16	0.62	3.17					
7/18/00	0.38	4.00	9.91	4.5	1.9	24.3	4.5-5.0	
2se	0.20	1.13	4.71					
8/23/00	0.42	3.02	12.90	4.3	3.2	23.9	4.0	
2se	0.24	0.82	4.69					
6/22/01	0.33	1.93	12.52	2.1	2.9	20.8	4.0-4.5	
2se	0.19	0.81	4.47					
7/24/01	0.38	2.42	13.57	14.4	2.3	26.9	4	
2se	0.24	1.37	5.15					
8/23/01	0.37	3.30	12.93	3.5	3.4	24.7	4.0-4.5	
2se	0.24	1.16	4.29					
7/2/02	0.38	4.41	24.2	-	3.1	26.1	4.5	
2se	0.12	1.73	20.0					
8/8/02	0.62	3.48	17.5	5.1	2.2	23.7	3	
2se	0.24	1.06	10.6					

Changes in milfoil biomass appeared related to herbivores during some periods in 3 lakes: Auburn, Otter and Smith's Bay. No changes associated with herbivores were seen at Cedar Lake. Herbivores were found at a very low density in Cedar Lake (Table 5). Caterpillars were rarely found and milfoil weevil densities rarely exceeded $5/m^2$. Adult milfoil weevils were extremely rare and it is possible that some larvae were actually *Phytobius leucogaster* larvae. *Phytobius* adults were found at Cedar and although they and their larvae are restricted to flowering stalks the larvae are indistinguishable from *Euhrychiopsis* and thus some *Phytobius* larvae may have been misidentified as *Euhrychiopsis*. The low density of herbivores and lack of clear declines of Eurasian watermilfoil at Cedar Lake indicates that herbivores are having no effect on the milfoil. Cage experiments reported in previous reports and in Ward (2002) indicate that high densities of sunfish are limiting herbivores at Cedar Lake. DNR Fisheries surveys indicate sunfish densities exceeding 100/trapnet.

Herbivores may have influenced milfoil density at Lake Auburn (Fig. 2). Weevil densities exceeded $100/m^2$ in July 1994 (Table 5) and Eurasian watermilfoil was around 1500 g/m^2. Weevil densities were much lower in 1995 ($< 10/m^2$) when Eurasian watermilfoil increased to over 5000 g/m^2 (Fig. 2). In 1996-1997 weevil densities increased and milfoil declined. Although weevil densities in 1998-1999 were very low, milfoil density remained low until it started to increase in 2000 with low weevil densities. In 2003, milfoil again declined following weevil densities of $20/m^2$ (Fig. 2).

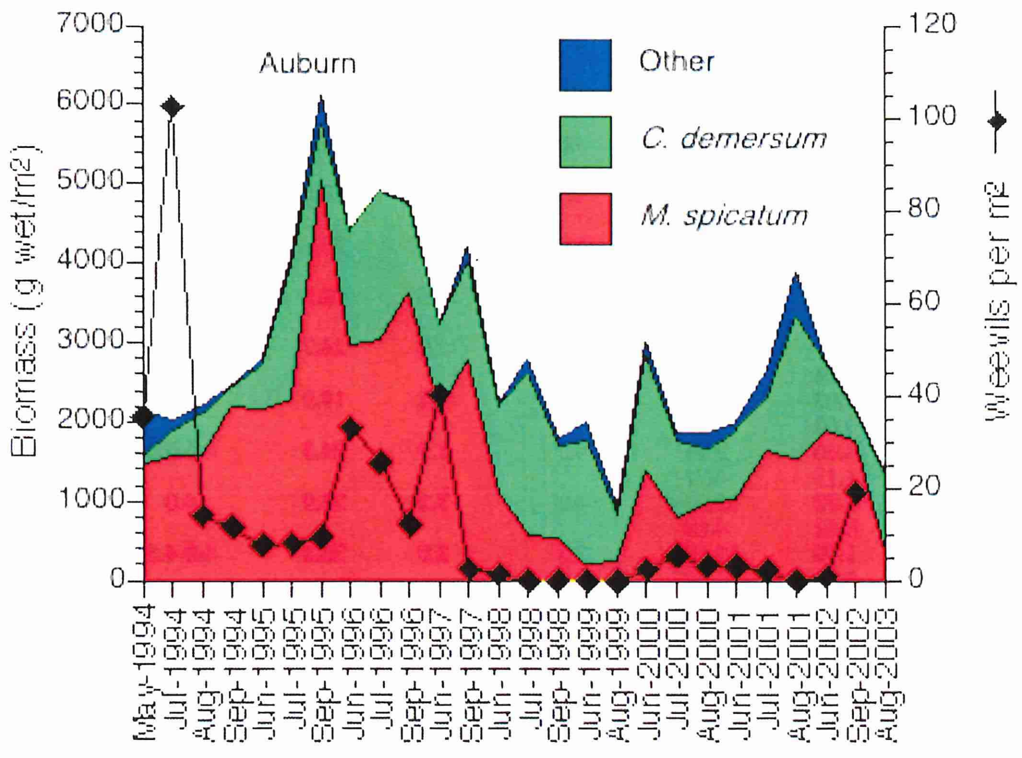

Fig. 2. Milfoil, coontail and other plant biomass (g wet/m^2) and weevil densities (N/m^2) at Lake Auburn as determined from biomass samples.

Densities of caterpillars were always low, generally < $5/m^2$ (Table 5). As discussed in the weevil survey section below, weevils disappeared from mid-summer 1998 until spring of 2000. Sunfish densities in Auburn exceeded 110/trapnet in 2000 and 86/trapnet in 1995. Herbivores may have facilitated the decline and suppression of milfoil at Lake Auburn but clearly were unable to have a sustained effect or maintain high densities for several years in a row.

Overall densities of herbivores were lower at Smith's Bay (Table 5), but do appear to have suppressed the plants in the shallow sites. Weevil densities were high in 1994 and Eurasian declined from a peak of over 5000 g/m^2 (Fig 3). Milfoil increased with lower weevil densities but increasing weevil densities were followed by milfoil suppression. The main effects were at the shallowest two sets of stations (100 and 200m from shore at 1.5 and 2m depth respectively) where weevil densities were highest (Fig. 4). Weevils were rarely found at the deepest site (4.5m) and abundances were very low at the 2 intermediate sites. At the shallowest stations, Eurasian watermilfoil was suppressed to <10% of plant biomass after 1996 and northern watermilfoil became common. Thus milfoil weevils appeared to control milfoil at the shallowest two sites in water ≤2m depth but not at deeper sites in Smith's Bay.

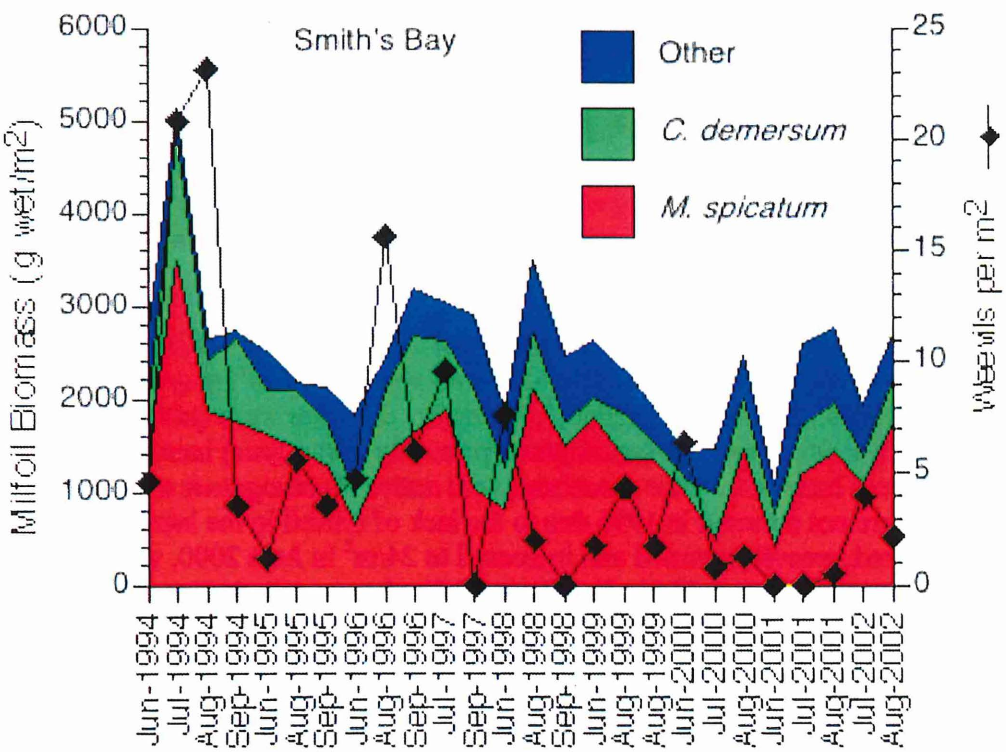

Fig. 3. Milfoil, coontail and other plant biomass (g wet/m^2) and weevil densities (N/m^2) at Smith's Bay as determined from biomass samples.

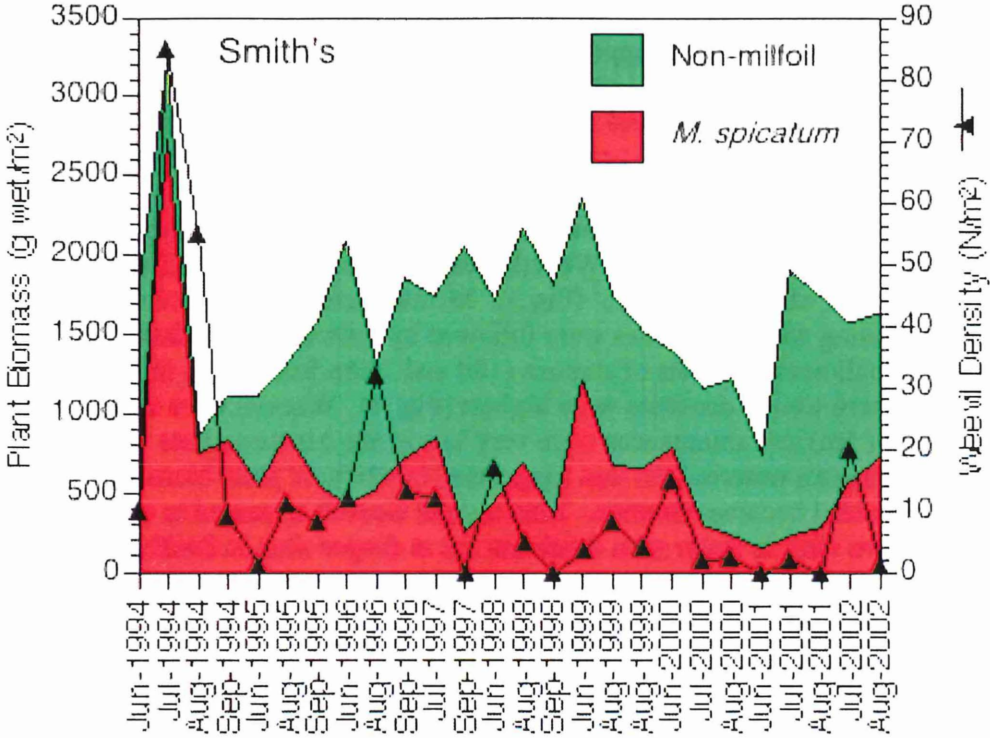

Fig. 4. Milfoil and non-milfoil plant biomass (g wet/m^2) and weevil densities (N/m^2) at the two shallowest stations (1.5 and 2m depth) at Smith's Bay as determined from biomass samples.

The first milfoil decline at Otter Lake, over the winter of 1995-1996, was likely due to winterkill (see above), however, moderate densities of milfoil weevils (12/m^2) may have contributed stress to the plants. Prior to the decline, Lepidoptera densities were quite low. After the milfoil decline in 1996, density of Lepidoptera (primarily *Parapoynx*) increased dramatically (Fig. 5). These herbivores were associated with native *Potamogeton*s and *Zosterella* and weevils were not detected in 1996 due to the lack of milfoil in the lake. As the milfoil slowly recovered, weevils returned and increased to 24/m^2 in June 2000, when milfoil had increased to over 2500 g/m^2 (Table 5). The milfoil subsequently declined that summer and remained suppressed through 2002 (Fig. 5). With the decrease in milfoil and increase in native plants Lepidoptera again became more abundant. Milfoil increased in 2003 with lower densities of milfoil weevils. The milfoil weevil caused extensive damage to milfoil in 2000-2002 and appeared to be the cause of the decline in that period. Aquatic lepidopterans may help suppress the milfoil during times of low density but were most abundant when there was little milfoil but numerous other plants, which they prefer. Sunfish densities in Otter Lake were quite low in 2000-2002 due to winterkills (<2 per trapnet) and were low in previous surveys (3-13/trapnet).

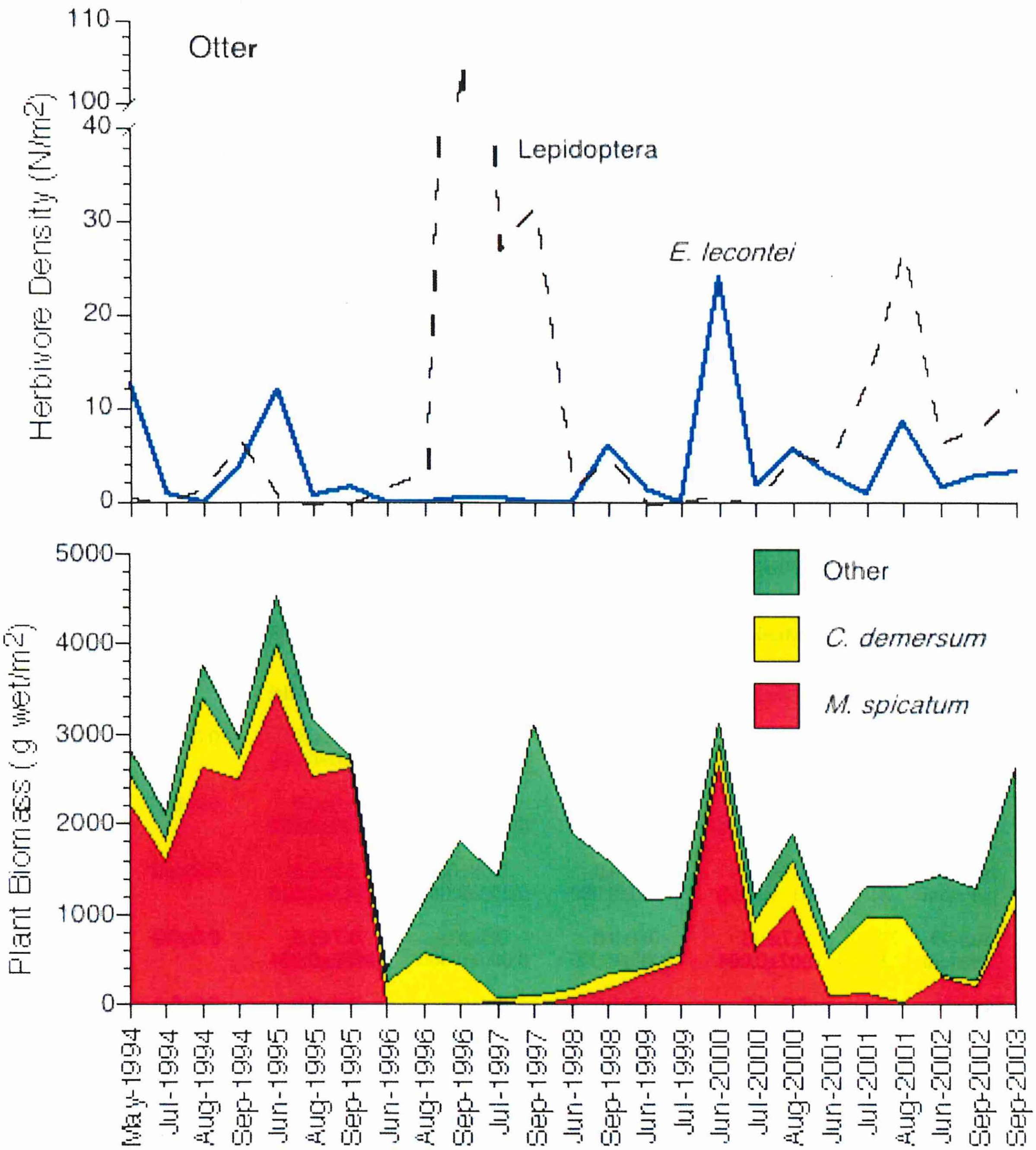

Fig. 5. Milfoil and non-milfoil plant biomass (g wet/m^2) and herbivore (milfoil weevils and Lepidoptera) densities (N/m^2) at Otter Lake as determined from biomass samples.

Table 5. Density (N/m^2 ± 2 SE and N per stem ± 2SE) of *Euhrychiopsis lecontei* larvae, pupae and adults, *Acentria ephemerella* and *Parapoynx* at the four permanent transect sites, 1994-2002. *Parapoynx* were not enumerated before 1996. A stem is a basal milfoil stem emerging from the sediment; estimates per stem do not include samples without milfoil and because caterpillars occurred often without milfoil, per stem estimates are not reported for them.

Cedar	Weevil	Larvae	Pupae	Adults	Total *E.l.*	*Acentria*	*Parapoynx*
Date	n	N/m^2	N/m^2	N/m^2	N/m^2	N/m^2	
May-94	11	5.5± 10.9	0.0± 0.0	0.9± 1.8	6.4± 10.9	0.0± 0.0	
per stem	0	–	–	–	–		
Jul-94	14	4.3± 8.6	1.4± 2.9	1.4± 2.9	7.1± 14.3	0.0± 0.0	
	0	–	–	–	–		
Aug-94	11	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Sep-94	17	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Jun-95	18	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Aug-95	10	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Sep-95	17	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Jun-96	29	0.3± 0.7	0.0± 0.0	0.0± 0.0	0.3± 0.7	0.0± 0.0	0.0±0.0
per stem	25	0.010±0.020	0.000±0.000	0.000±0.000	0.010±0.020		
Aug-96	21	0.0± 0.0	0.5± 1.0	0.5± 1.0	1.0± 1.9	0.0± 0.0	0.0±0.0
per stem	21	0.000±0.000	0.002±0.004	0.002±0.004	0.004±0.008		
Sep-96	23	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0±0.0
per stem	24	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jul-97	28	0.0±0.0	0.0±0.0	0.4±0.7	0.4±0.7	0.4±0.7	0.0±0.0
per stem	28	0.000±0.000	0.000±0.000	0.002±0.003	0.002±0.003		
Sep-97	26	0.8±1.1	0.0±0.0	0.4±0.8	1.2±1.3	0.0±0.0	0.0±0.0
per stem	26	0.012±0.016	0.000±0.000	0.002±0.003	0.013±0.019		
Jun-98	31	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
per stem	30	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-98	28	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.4±0.7	0.0±0.0
per stem	24	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-99	26	1.9±2.5	0.0±0.0	0.38±0.77	2.3±2.6	0.0±0.0	0.0±0.0
per stem	24	0.011±0.013	0.000±0.000	0.003±0.006	0.013±0.013		
Aug-99	27	0.7±1.5	0.0±0.0	0.0±0.0	0.7±1.5	0.0±0.0	0.0±0.0
per stem	26	0.002±0.004	0.000±0.000	0.000±0.000	0.002±0.004		
Jun-00	26	7.7±6.8	0.8±1.5	0.4±0.8	8.8±7.8	0.0±0.0	0.0±0.0
per stem	25	0.035±0.031	0.003±0.005	0.001±0.002	0.039±0.034		
Aug-00	27	3.3±3.2	0.0±0.0	0.0±0.0	3.3±3.2	0.7±1.0	0.0±0.0
per stem	25	0.023±0.023	0.000±0.000	0.000±0.000	0.023±0.023		
Jun-01	28	0.0±0.0	1.1±2.1	2.1±4.3	3.2±6.4	0.0±0.0	0.0±0.0
per stem	20	0.000±0.000	0.017±0.033	0.033±0.067	0.050±0.100		
Aug-01	24	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
per stem	12	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jul-02	18	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
per stem	16	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Aug-02	29	1.4±1.3	0.0±0.0	0.0±0.0	1.4±1.3	0.0±0.0	0.3±0.7
per stem	23	0.010±0.010	0.000±0.000	0.000±0.000	0.010±0.010		

Table 5. Continued.

Auburn Date	Weevil n	Larvae N/m^2	Pupae N/m^2	Adults N/m^2	Total *E.l.* N/m^2	*Acentria* N/m^2	*Parapoynx*
May-94	9	27.8 ±27.4	1.1± 2.2	6.7± 8.8	35.6± 36.5	1.1± 2.2	
per stem	9	0.134±0.103	0.002±0.004	0.018±0.020	0.154±0.106		
Jul-94	16	58.8± 21.1	12.5± 9.6	31.3± 14.0	102.5± 36.7	6.3± 7.7	
per stem	16	0.217±0.092	0.034±0.034	0.084±0.036	0.335±0.127		
Aug-94	15	8.7± 7.5	2.0± 2.9	3.3± 3.7	14.0± 9.5	0.7± 1.3	
per stem	15	0.031±0.025	0.003±0.005	0.008±0.008	0.042±0.030		
Sep-94	18	1.7± 3.3	2.2± 2.6	7.8± 7.8	11.7± 11.8	3.9± 3.3	
per stem	18	0.002±0.004	0.006±0.008	0.014±0.012	0.022±0.019		
Jun-95	30	6.0± 4.0	0.7± 0.9	1.0± 1.1	7.7± 2.7	0.3± 0.7	
per stem	21	0.070±0.043	0.003±0.006	0.011±0.015	0.085±0.056		
Jul-95	15	2.0± 2.1	0.7± 1.3	5.3± 5.5	8.0± 3.8	0.0± 0.0	
per stem	14	0.006±0.009	0.000±0.000	0.032±0.039	0.038±0.042		
Sep-95	16	2.5± 2.2	3.1± 3.5	3.8± 4.0	9.4± 3.4	1.3± 1.7	
per stem	11	0.140±0.194	0.049±0.090	0.103±0.180	0.292±0.385		
Jun-96	30	31.0± 17.8	2.0± 2.0	0.0± 0.0	33.0± 19.5	0.3± 0.7	0.0± 0.0
per stem	27	0.729±1.179	0.080±0.148	0.000±0.000	0.809±1.326		
Jul-96	25	9.2± 15.2	3.6± 2.6	12.8± 6.3	25.6± 17.9	1.6±1.5	0.8±1.1
per stem	23	0.029±0.043	0.020±0.021	0.048±0.027	0.096±0.061		
Sep-96	30	6.7± 4.3	2.3± 1.6	3.0± 2.7	12.0± 6.5	0.7±0.9	5.7± 4.4
per stem	29	0.048±0.053	0.007±0.005	0.011±0.010	0.065±0.055		
Jun-97	30	35.7±19.6	0.3±0.7	4.3±5.9	40.3±24.3	0.7±1.3	0.0±0.0
per stem	27	0.201±0.126	0.001±0.003	0.022±0.027	0.224±0.144		
Sep-97	30	0.3±0.7	0.0±0.0	1.7±1.4	2.0±1.5	1.7±2.7	2.3±2.8
per stem	29	0.001±0.001	0.000±0.000	0.007±0.007	0.008±0.008		
Jun-98	27	1.0±1.1	0.0±0.0	0.3±0.7	1.3±1.3	1.0±2.0	0.0±0.0
per stem	27	0.005±0.005	0.000±0.000	0.001±0.003	0.006±0.006		
Jul-98	28	0.0±0.0	0.0±0.0	0.0±0.0	0.±0.0	0.7±1.0	0.0±0.0
per stem	24	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-98	30	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.7
per stem	28	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-99	27	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.7	0.0±0.0
per stem	19	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Aug-99	27	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
per stem	19	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-00	26	0.8±1.1	0.0±0.0	1.5±1.4	2.3±2.0	0.0±0.0	0.0±0.0
per stem	23	0.004±0.005	0.000±0.000	0.007±0.007	0.010±0.009		
Jul-00	28	1.6±2.5	0.4±0.8	3.6±3.6	5.4±5.5	0.0±0.0	0.0±0.0
per stem	21	0.009±0.014	0.004±0.008	0.027±0.025	0.039±0.038		
Aug-00	28	1.1±2.1	0.0±0.0	2.1±2.4	3.2±4.4	0.0±0.0	2.1±3.1
per stem	27	0.011±0.022	0.000±0.000	0.024±0.028	0.035±0.047		
Jun-01	29	0.3±0.7	2.4±2.6	0.7±1.0	3.4±2.7	0.0±0.0	0.0±0.0
per stem	24	0.003±0.006	0.023±0.029	0.008±0.012	0.034±0.030		
Jul-01	30	0.7±0.9	0.3±0.7	1.0±1.1	2.0±1.5	0.0±0.0	0.0±0.0
per stem	25	0.011±0.015	0.002±0.003	0.007±0.008	0.019±0.016		
Aug-01	30	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.3±4.0	5.0±6.0
per stem	19	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		

Table 5. Continued.

Date	Weevil n	Larvae N/m^2	Pupae N/m^2	Adults N/m^2	Total *E.l.* N/m^2	*Acentria* N/m^2	*Parapoynx*
Auburn Cont:							
Jun-02	30	0.37±0.7	0.07±0.0	0.37±0.7	0.77±0.9	0.07±0.0	0.07±0.0
per stem	29	0.003±0.006	0.000±0.000	0.001±0.002	0.004±0.006		
Sep-02	27	4.87±3.3	3.07±3.3	11.97±7.6	18.97±11.5	3.07±2.6	0.47±0.0
per stem	27	0.021±0.015	0.009±0.010	0.045±0.028	0.076±0.044		
Otter							
May-94	20	12.5± 10.2	0.0± 0.0	0.0± 0.0	12.5± 10.2	0.5± 1.0	
per stem	20	0.047±0.038	0.000±0.000	0.000±0.000	0.047±0.038		
Jul-94	24	0.4± 0.9	0.0± 0.0	0.4± 0.9	0.8± 1.2	0.0± 0.0	
	24	0.001±0.002	0.000±0.000	0.001±0.003	0.002±0.003		
Aug-94	14	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	1.4± 2.9	
	14	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-94	8	0.0± 0.0	1.3± 2.5	2.5± 3.3	3.8± 3.7	6.3± 5.3	
	7	0.000±0.000	0.003±0.007	0.013±0.022	0.016±0.021		
Jun-95	27	5.9± 5.1	2.6± 3.3	3.3± 3.4	11.9± 9.0	0.4± 0.7	
	26	0.033±0.030	0.021±0.034	0.022±0.020	0.076±0.071		
Aug-95	15	0.0± 0.0	0.0± 0.0	0.7± 1.3	0.7± 1.3	0.0± 0.0	
	1	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-95	18	0.6± 1.1	0.0± 0.0	1.1± 2.2	1.7± 2.4	0.0± 0.0	
	1	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-96	25	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.8± 1.6	0.8±1.6
	5	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Aug-96	26	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.8± 1.1	2.3± 2.0
	2	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-96	27	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	4.4± 3.6	100.4±24.5
	0	–	–	–	–		
Jul-97	26	0.4±0.8	0.0±0.0	0.0±0.0	0.4±0.8	6.2± 3.9	20.8±20.5
	3	0.083±0.167	0.000±0.000	0.000±0.000	0.083±0.167		
Sep-97	27	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.5±1.8	30.0±13.8
	1	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-98	27	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.1±1.6	0.4±0.7
	13	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-98	27	4.1±4.3	0.0±0.0	1.9±3.0	5.9±5.1	0.0±0.0	4.4±5.4
	16	0.206±0.219	0.000±0.000	0.049±0.084	0.255±0.223		
Jun-99	22	1.4±2.0	0.0±0.0	0.0±0.0	1.4±2.0	0.0±0.0	0.0±0.0
	20	0.030±0.050	0.000±0.000	0.000±0.000	0.030±0.050		
Jul-99	26	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	26	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-00	27	14.4±14.8	4.8±4.3	4.8±3.9	24.1±20.4	0.0±0.0	0.4±0.7
	27	0.092±0.093	0.029±0.037	0.028±0.027	0.150±0.131		
Jul-00	27	1.1±1.6	0.0±0.0	0.7±1.5	1.9±3.0	0.0±0.0	0.0±0.0
	27	0.019±0.030	0.000±0.000	0.015±0.030	0.033±0.059		
Aug-00	27	4.1±4.8	0.0±0.0	1.5±1.4	5.6±5.7	1.9±1.5	3.3±2.4
	27	0.064±0.074	0.000±0.000	0.011±0.012	0.076±0.083		

Table 5. Continued.
Otter Continued:

Date	Weevil n	Larvae N/m²	Pupae N/m²	Adults N/m²	Total *E.l.* N/m²	*Acentria* N/m²	*Parapoynx* N/m²
Jun-01	27	1.1±2.2	0.4±0.7	2.2±3.3	3.7±4.3	4.1±3.6	0.7±1.5
per stem	21	0.024±0.034	0.005±0.010	0.083±0.131	0.111±0.134		
Jul-01	25	0.0±0.0	0.0±0.0	0.8±1.6	0.8±1.6	0.4±0.8	13.2±9.5
per stem	4	0.000±0.000	0.000±0.000	0.250±0.500	0.250±0.500		
Aug-01	23	5.7±6.6	0.0±0.0	0.4±0.9	6.1±7.4	2.6±3.8	27.0±11.6
per stem	0	.-	.-	.-.	.-		
Jun-02	27	1.1±1.2	0.7±1.5	0.7±1.0	1.5±1.8	3.3±2.4	3.0±2.8
per stem	20	0.078±0.109	0.007±0.013	0.006±0.009	0.091±0.109		
Sep-02	26	1.5±1.8	0.4±0.8	0.8±1.1	2.7±2.1	2.7±2.4	5.0±5.0
per stem	26	0.038±0.046	0.005±0.010	0.019±0.027	0.063±0.051		
Smith's Bay							
Jun-94	13	3.8± 5.3	0.0± 0.0	0.8± 1.5	4.6± 6.6	0.0± 0.0	
per stem	12	0.020±0.030	0.000±0.000	0.005±0.010	0.025±0.040		
Jul-94	11	12.3± 13.0	6.9± 8.0	1.5± 2.1	20.8± 20.9	0.8± 1.5	
	13	0.064±0.083	0.038±0.052	0.006±0.009	0.108±0.137		
Aug-94	16	18.0± 15.0	3.1± 4.0	1.9± 2.7	23.1± 20.2	0.6± 1.3	
	15	0.104±0.079	0.019±0.022	0.010±0.015	0.133±0.109		
Sep-94	14	0.0± 0.0	1.4± 2.9	2.1± 2.3	3.6± 4.5	0.0± 0.0	
	14	0.000±0.000	0.003±0.006	0.013±0.020	0.016±0.022		
Jun-95	25	0.4± 0.8	0.0± 0.0	0.8± 1.1	1.2± 1.3	0.0± 0.0	
	14	0.001±0.003	0.000±0.000	0.027±0.048	0.028±0.047		
Aug-95	25	4.0± 4.3	1.2± 1.8	0.4± 0.8	5.6± 5.3	0.0± 0.0	
	9	0.080±0.096	0.000±0.000	0.007±0.015	0.087±0.107		
Sep-95	25	0.8± 1.1	2.0± 3.3	0.8± 1.1	3.6± 5.0	0.0± 0.0	
	15	0.010±0.014	0.025±0.039	0.013±0.019	0.048±0.061		
Jun-96	25	4.8± 5.8	0.0± 0.0	0.0± 0.0	4.8± 5.8	5.2± 8.8	0.0± 0.0
	20	0.037±0.043	0.000±0.000	0.000±0.000	0.037±0.043		
Aug-96	25	12.4± 10.0	1.2± 1.8	2.0± 2.0	15.6± 10.5	0.0± 0.0	1.6± 2.5
	24	0.107±0.084	0.006±0.008	0.015±0.015	0.127±0.087		
Sep-96	25	1.2± 1.8	2.0± 2.0	2.8± 3.4	6.0± 5.3	0.8± 1.1	0.0± 0.0
	24	0.005±0.007	0.009±0.009	0.014±0.015	0.028±0.022		
Jul-97	25	5.2±4.3	0.4±0.8	4.0±3.7	9.6±6.9	0.0± 0.0	0.8±1.6
	21	0.049±0.053	0.003±0.005	0.043±0.049	0.094±0.094		
Sep-97	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.4±0.8	0.0± 0.0
	21	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-98	25	7.2±7.2	0.4±0.8	0.0±0.0	7.6±7.6	1.2±1.8	0.0±0.0
	21	0.052±0.054	0.002±0.005	0.000±0.000	0.054±0.055		
Aug-98	25	1.2±1.8	0.0±0.0	0.8±1.1	2.0±2.0	0.0±0.0	0.0±0.0
	20	0.017±0.023	0.000±0.000	0.002±0.005	0.019±0.023		
Sep-98	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.4±0.8
	19	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-99	22	0.9±1.3	0.0±0.0	0.9±1.3	1.8±2.1	0.9±1.3	0.0±0.0
	22	0.047±0.091	0.000±0.000	0.047±0.091	0.094±0.182		
Jul-99	25	2.4±4.8	0.8±1.1	1.2±1.3	4.4±4.9	0.0±0.0	1.2±1.5
	21	0.000±0.000	0.002±0.003	0.014±0.024	0.017±0.024		
Aug-99	23	0.9±1.2	0.0±0.0	0.9±1.2	1.7±2.0	0.0±0.0	0.0±0.0
	22	0.005±0.007	0.000±0.000	0.007±0.010	0.012±0.015		

Table 5. Continued.
Smith's Bay Continued:

Date	Weevil n	Larvae N/m²	Pupae N/m²	Adults N/m²	Total *E.l.* N/m²	*Acentria* N/m²	*Parapoynx* N/m²
Jun-00	22	3.6±4.1	0.9±1.8	1.8±1.7	6.4±5.5	1.4±2.0	0.0±0.0
	20	0.027±0.035	0.007±0.014	0.008±0.009	0.042±0.042		
Jul-00	24	0.0±0.0	0.0±0.0	0.8±1.7	0.8±1.7	0.0±0.0	0.0±0.0
	19	0.000±0.000	0.000±0.000	0.009±0.018	0.009±0.018		
Aug-00	23	1.3±1.4	0.0±0.0	0.0±0.0	1.3±1.4	0.0±0.0	1.7±2.4
	21	0.009±0.010	0.000±0.000	0.000±0.000	0.009±0.010		
Jun-01	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.4±0.8	0.0±0.0
per stem	13	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jul-01	24	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
per stem	17	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Aug-01	20	0.0±0.0	0.0±0.0	0.5±1.0	0.5±1.0	0.0±0.0	0.0±0.0
per stem	14	0.000±0.000	0.000±0.000	0.002±0.005	0.002±0.005		
Jul-02	25	5.6±4.8	0.8±1.1	1.6±2.2	4.0±5.0	0.0±0.0	0.0±0.0
per stem	19	0.117±0.210	0.001±0.002	0.113±0.210	0.231±0.420		
Aug-02	24	1.4±2.5	0.1±0.0	0.9±1.2	2.2±2.7	0.5±0.8	0.1±0.0
per stem	19	0.004±0.009	0.000±0.000	0.009±0.012	0.013±0.014		

Cenaiko Lake:

Cenaiko Lake provides a clear example of a weevil induced decline and also illustrates the role of sunfish in herbivore densities and milfoil control. Milfoil biomass declined significantly in 1996 with high densities of weevils (Newman and Biesboer 2001). Milfoil increased in summer 1998 but was again controlled by weevils and remained suppressed (<10% of total biomass) through 2001 (Fig 6). Milfoil increased to nearly 70 g/m^2 and more than 30% of total biomass in 2002 (Table 6). Milfoil biomass continued to increase at Cenaiko Lake in 2003 to 170 g/m^2, exceeding the previous peak biomass (123 g dry/m^2) found in 1996 at the start of the decline (Fig 6). Milfoil became the dominant plant, composing almost 70% of total plant biomass in late July 2003, the highest percentage since the decline in 1996. Herbivore densities were very low in 2001-2002 (Table 7). Native plant biomass remained relatively high and similar to 2000-2001 at 120g dry/m^2, and the mean and total number of species remained similar to previous years. Good water clarity in 2003 (Secchi of 4.8m in late July) probably helped maintain some native plants while enhancing milfoil growth, in contrast to 2002 when poor water clarity associated with summer rains may have suppressed the plant community (Table 8). However, low densities of herbivores since 2002 (only 2 weevil eggs detected in 2003; see below) are failing to control the milfoil.

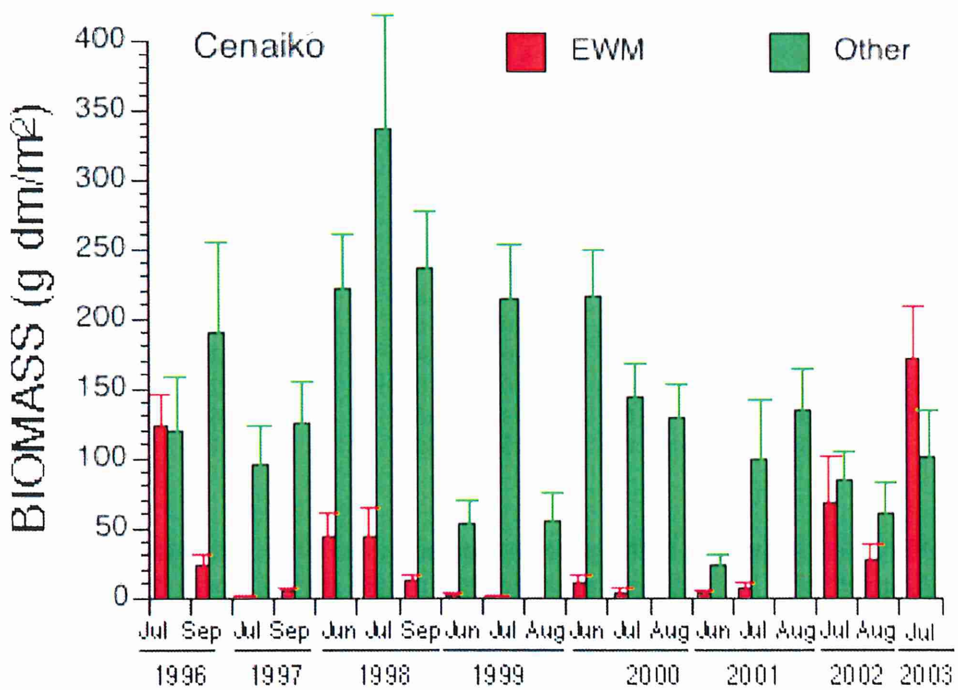

Fig. 6. Biomass (g dm/m^2 +1SE) of Eurasian watermilfoil and all other plants at Cenaiko Lake 1996-2003.

Table 6. Biomass (g dry/m^2) of all plants (Total), Eurasian watermilfoil (MSP), the dominant plants (coontail (CRT), *Zosterella* (= *Heteranthera*) *dubia* (ZOS), *Potamogeton zosteriformis* (PZS), *Chara* (CHA) and *Potamogeton amplifolius* (PAM)), non-milfoil biomass (NAT), total (TN) and mean number of species (N Sp) and mean percentage of biomass that was Eurasian watermilfoil in Cenaiko Lake 1999-2003. N=17-27 samples per date. In July and August 2001, *Potamogeton nodosus* was present at densities of 36 and 19 g dry/m^2 and in August 2002 at 50 g/m^2. In 2002 *P. pectinatus* was present at 2-3 g/m^2. In 2003, *P. pectinatus* was present at 2g/m^2.

Date	Total	MSP	CRT	PZS	ZOS	CHA	PAM	TN	N Sp.	NAT	%MSP
6/24/99	53.7	1.3	32.2	0.2	3.0	0.5	12.3	11	1.9	52.4	7.9%
1 S.E.	17.0	0.9	12.0	0.2	2.5	0.4	10.7		0.2	17.1	5.2%
8/2/99	214.6	1.1	124.5	0.0	26.7	0.0	34.1	10	2.6	213.5	1.0%
1 S.E.	40.1	0.8	37.5	0.0	9.7	0.0	23.6		0.2	40.2	0.7%
8/26/99	55.0	0.0	30.2	0.1	5.0	0.0	6.7	5	1.5	55.0	0.0%
1 S.E.	20.1	0.0	20.1	0.1	3.4	0.0	4.4		0.1	20.1	0.0%
6/29/00	225.9	10.0	123.9	0.0	16.3	46.0	19.8	9	2.1	215.9	3.1%
1 SE	34.1	5.2	31.2	0.0	8.2	21.1	14.3		0.2	33.1	1.7%
7/20/00	146.8	3.7	86.4	0.0	19.5	14.5	18.3	8	2.4	143.2	8.4%
1 SE	23.6	2.2	22.5	0.0	10.1	9.4	11.8		0.3	24.1	5.1%
8/30/00	134.5	0.1	89.4	34.5	0.0	8.0	1.7	8	1.8	129.4	0.1%
1 SE	22.0	0.1	23.5	14.9	0.0	7.3	1.5		0.2	22.8	0.1%
6/26/01	25.5	2.8	17.2	0.6	0.0	0.0	0.6	7	1.4	22.7	3.5%
1 SE	8.5	2.8	7.9	0.3	0.0	0.0	0.6		0.4	8.0	3.3%
7/30/01	105.4	6.8	59.5	0.0	0.0	0.0	0.0	7	1.1	98.6	7.1%
1 SE	43.1	4.0	26.1	0.0	0.0	0.0	0.0		0.3	42.6	4.4%
8/27/01	133.6	0.0	98.8	1.0	0.0	0.0	8.8	6	1.0	133.6	4.0%
1 SE	29.6	0.0	27.3	0.5	0.0	0.0	6.4		0.1	29.6	4.0%
7/1/02	152.4	67.7	74.6	4.0	0.0	0.0	0.0	5	2.2	84.8	19.4%
1 SE	44.5	34.3	21.8	3.2	0.0	0.0	0.0		0.2	20.7	8.7%
8/27/02	87.8	26.9	51.3	0.1	0.0	0.0	0.0	6	1.8	60.9	36.8%
1 SE	21.1	11.3	22.5	0.1	0.0	0.0	0.0		0.2	22.0	11.3%
7/28/03	271.2	170.7	69.9	9.6	0.0	4.4	15.1	6	2.6	100.4	70.4%
1 SE	53.2	37.1	22.3	9.3	0.0	3.3	15.1		0.1	34.2	7.1%

Fish surveys (DNR Lake Survey) in 1992, prior to the decline in 1996, indicated a high density of sunfish (95/trapnet set). In 1998, just after the decline and during a period of high weevil densities, sunfish density had dropped to 5/trapnet. Fish surveys in 2002 indicated a density of sunfish of 25/trapnet, 5 times higher than in 1998. As noted below sunfish appear to be limiting weevil and herbivore densities in many of our lakes. Although preliminary analysis of fish survey data from 2003 indicated only 15 sunfish/trapnet, the higher sunfish density in 2002 may have effectively eliminated the milfoil weevil from Cenaiko during 2003 (see below). It is not known how long natural recolonization would take to reestablish a viable weevil population if sunfish density would further decline.

Table 7. Density (N/m^2 ± 2 SE and N per stem) of *Euhrychiopsis lecontei* (*E.l.*) larvae, pupae and adults, and *Acentria ephemerella* and *Parapoynx* sp. at Cenaiko Lake in 1996-2002. Densities per stem were only calculated for samples with Eurasian watermilfoil and because the caterpillars often occurred in samples with no milfoil their densities per stem were not calculated. A stem is a basal milfoil stem emerging from the sediment. Samples with no plants were not included in herbivore density estimates.

Date	Weevil n	Larvae N/m^2	Pupae N/m^2	Adults N/m^2	Total *E.l.* N/m^2	*Acentria* N/m^2	*Parapoynx* N/m^2
7/22/96	29	48.6± 25.2	22.8± 10.8	31.7± 13.6	103.1± 41.9	18.3± 7.7	1.0± 1.5
per stem	26	0.923±1.292	0.337±0.458	0.381±0.280	1.640±1.972		
9/5/96	21	2.9± 2.4	1.0± 1.3	4.3± 4.3	8.1± 5.6	31.9± 20.2	0.0± 0.0
per stem	8	0.229±0.259	0.008±0.017	0.417±0.516	0.654±0.721		
7/16/97	26	1.5±1.8	0.0±0.0	0.0±0.0	1.5±1.8	8.8±5.8	0.0±0.0
per stem	3	0.389±0.401	0.000±0.000	0.000±0.000	0.389±0.401		
9/17/97	24	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	32.1±19.6	1.7±2.0
per stem	6	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
6/16/98	25	0.4±0.8	0.0±0.0	0.0±0.0	0.4±0.8	17.6±9.1	0.4±0.8
per stem	15	0.004±0.009	0.000±0.000	0.000±0.000	0.004±0.009		
7/29/98	25	0.0±0.0	0.0±0.0	0.8±1.6	0.8±1.6	1.6±1.5	0.4±0.8
per stem	12	0.000±0.000	0.000±0.000	0.019±0.037	0.019±0.037		
9/14/98	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	6.4±4.5	21.6±19.8
per stem	3	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
6/24/99	26	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	16.9±10.3	0.0±0.0
per stem	3	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
8/2/99	24	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.0±1.1	0.0±0.1
per stem	3	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
8/26/99	23	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	6.5±5.4	0.0±0.0
per stem	0	-	-	-	-		
06/29/00	22	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	69.1±43.2	0.0±0.0
per stem	6	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
07/20/00	22	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	32.0±16.1	3.0±5.0
per stem	7	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
08/30/00	21	0.5±1.0	0.0±0.0	0.0±0.0	0.5±1.0	12.9±9.4	4.3±8.6
per stem	7	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
6/26/01	20	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3.5±4.9	0.0±0.0
per stem	1	0.000±.	0.000±.	0.000±.	0.000±.		
7/30/01	21	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	4.8±4.3	0.0±0.0
per stem	3	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
8/27/01	19	0.5±1.1	0.0±0.0	0.0±0.0	0.5±1.1	0.0±0.0	0.0±0.0
per stem	0	-	-	-	-		
7/1/02	15	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	5.3±5.1	0.0±0.0
per stem	7	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
8/27/02	16	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.3±1.7	0.6±1.2
per stem	8	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		

Table 8. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium and water column characteristics in 1996-2003 at Cenaiko Lake. Sediment samples were collected from shallow, moderate and deep stations along transects 1, 2 and 3 (n=9).

Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/m^3)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Plant Limit (m)
7/22/96	1.23	0.60	1.5%	1.34	5.0	25.4	4.5-5.0	3.4
2se	0.22	0.54	0.5%					
9/5/96	1.22	0.67	2.4%	5.61	4.0	25.7	5.0	3.4
2se	0.23	0.40	1.1%					
7/16/97	1.10	1.63	2.5%	4.54	2.3	27.6	3.5	3.0
2se	0.20	0.67	0.6%					
9/17/97	0.96	2.87	2.5%	1.60	2.3	21.3	2.0-2.5	3.0
2se	0.18	1.65	0.5%					
6/16/98	0.98	2.37	2.2%	2.41	3.8	23.7	5.5-6.0	3.4
2se	0.18	0.66	0.5%					
7/29/98	0.97	4.98	2.3%	2.41	4.4	25.9	4.5-5.0	3.4
2se	0.16	2.31	0.7%					
9/14/98	1.12	6.08	1.7%	3.21	3.0	23.8	3.5-4.0	3.2
2se	0.12	4.90	0.5%					
6/24/99	1.12	1.12	1.76%	1.3	2.7	24.3	3.5-4.0	
2SE	0.24	0.24	0.82%					
8/2/99	1.14	2.09	1.29%	3.5	2.7	27.4	3.0-3.5	
2SE	0.17	0.78	0.40%					
8/26/99	1.22	4.20	1.30%	2.1	3.1	24.3	3.0-3.5.0	
2SE	0.14	1.27	0.45%					
6/29/00	1.08	1.11	2.31%	2.14	2.3	23.5	3.5	
2se	0.27	0.73	0.41%					
7/20/00	1.13	4.09	3.01%	3.47	1.6	23.2	2.0-2.5	
2se	0.35	.	1.57%					
8/30/00	1.25	3.27	2.43%	2.94	1.4	23.1	4.5-5.0	
2se	0.26	2.41	0.70%					
6/26/01	1.05	1.45	3.69%	4.3	1.3	25.2	2.5	
2se	0.28	0.75	3.66					
7/30/01	1.27	2.07	1.80%	4.5	0.9	26.9	1.5	
2se	0.23	0.65	0.59					
8/27/01	1.26	3.92	1.70%	17.6	2.3	25.6	4.5	
2se	0.21	2.08	0.60					
7/1/02	1.42	2.39	5.3	-	1.2	29.0	1.5-2.0	
2se	0.63	1.63	4.2					
8/27/02	1.51	2.57	7.8	4.0	3.8	24.6	4	
2se	0.24	1.41	2.2					
7/28/03	1.14	3.54	2.3	.	4.8	26.2	5.0	
2se	0.39	1.72	1.1					

Weevil surveys:

The biomass samples provide an estimate of herbivore densities, however, the samples are infrequent, some herbivores may be overlooked in the large plant samples and when milfoil density is low, relatively few milfoil stems may be sampled. We therefore conducted biweekly weevil surveys, which provide a better assessment of weevil populations and are less likely to miss weevils due to peaks and troughs in abundance through the life cycle. Weevil eggs are also enumerated. Biweekly weevil surveys were conducted in Lake Auburn, Cenaiko Lake, and Smiths Bay from 1999-2003 and Otter from 2000-2003. Results of 1998 and 1999 surveys in Auburn were presented in our previous report and are summarized here.

Weevil densities were highest at Cenaiko Lake in 1999, with a summer mean of 0.7/stem and almost 0.1 adults per stem (Table 9). Weevil densities at Cenaiko slowly declined over the next four years. In 2000, summer average weevil densities exceeded 0.3 per stem but this dropped below 0.1 per stem in 2002; only 2 weevil eggs were found in 2003 and no other life stages were detected (Table 9). *Acentria* and *Parapoynx* densities were also decreased in 2002 and 2003. As noted above, sunfish appear to be limiting weevil and herbivore densities in many of our lakes and Cenaiko Lake appears a prime example. Milfoil started to increase when mean summer density fell below 0.1 per stem (2002).

Lake Auburn illustrates that summer factors are limiting weevil densities. In May 1998 over 1 weevil per stem was found in Auburn but by mid-July no weevils were found in our surveys. No weevils were found the rest of 1998 and in all of 1999. However, weevils were found again in May 2000 (Table 9). Since then summer densities have averaged between 0.04 and 0.07 per stem, however, there were several months each year when no weevils were detected. Fish predation is likely limiting weevil populations and their reappearance in spring 2000 suggests recolonization from elsewhere. The large increase in adults in September 2002 suggests fall movement from elsewhere also. Although densities were not high in our samples in 2003, elsewhere in the lake adult densities were very high. Adult densities were so high that we collected weevils for stocking in Harriet and Hiawatha from Lake Auburn in 2003. *Acentria* and *Parapoynx* were rarely detected at Lake Auburn. High sunfish densities (110/trapnet in 2000) are likely suppressing herbivore densities at Auburn.

Biweekly surveys in Otter Lake show an increase from a summer long average of 0.16/stem in 2000 to 0.42/stem in 2001 (Table 9). There was too little milfoil in biomass samples in 2001-2003 to get good weevil estimates and the stem surveys are likely a better indication of density. Weevil densities during the main decline in June 2000 exceeded 0.4/stem. Weevils remained fairly abundant through 2003 but adult densities were lower in 2003 and the population appeared to be decreasing. *Acentria* and *Parapoynx* densities also decreased in 2002-2003 and neither were very abundant on the milfoil plants (densities <0.3 per stem). As noted above, the high herbivore densities were controlling the milfoil and low sunfish densities (2/trapnet in 2001 and 6/trapnet in 2002) permitted development of high herbivore populations at least through 2002.

Weevil densities in Smith's Bay were fairly high in 1999 and 2000 with summer means of 0.33 and 0.25/stem respectively. These surveys are conducted in the three shallowest stations (1.5-2.5m depth) where the milfoil has been controlled by herbivory. Weevil densities were low in 2001 (mean of 0.09) but increased in to > 0.1/stem 2002 and 2003. A few *Acentria* have been found at Smith's but *Parapoynx* were not detected. As noted above, the moderate and persistent densities of weevils at Smith Bay appear to be controlling milfoil at the shallowest two stations but not at deeper stations.

Table 9. Density of weevil life stages (per stem), total weevils per stem and density of the caterpillars *Acentria* (Acent) and *Parapoynx* (Parap) from the bi-weekly weevil surveys. Caterpillars were not enumerated in the 1999 samples.

Lake Date	Eggs	Larvae	Pupae	Adults	Total	Acent	Parap
Cenaiko							
6/10/99	1.0000	0.2500	0.0000	0.3500	1.6000	-	-
6/24/99	0.1333	0.0556	0.0000	0.0208	0.2097	-	-
7/9/99	0.2000	0.8500	0.2500	0.0000	1.3000	-	-
7/22/99	0.2909	0.2909	0.0909	0.0909	0.7636	-	-
8/2/99	0.1333	0.0000	0.0000	0.0533	0.1867	-	-
8/18/99	0.4854	0.3760	0.0417	0.1427	1.0458	-	-
9/2/99	0.0000	0.3472	0.0000	0.0519	0.3991	-	-
9/15/99	0.0000	0.0000	0.0000	0.0375	0.0375	-	-
Mean	0.2804	0.2712	0.0478	0.0934	0.6928		
5/16/00	0.1952	0.0229	0.0000	0.0000	0.2181	0.2762	0.0000
5/30/00	0.0397	0.0159	0.0069	0.0000	0.0625	0.1905	0.0000
6/13/00	0.1190	0.0883	0.0488	0.0756	0.3318	0.1584	0.0000
6/29/00	0.2476	0.0556	0.0397	0.0238	0.3667	0.0508	0.0000
7/11/00	0.3214	0.0347	0.0208	0.1141	0.4911	0.1141	0.0000
7/24/00	0.7393	0.0208	0.0069	0.1181	0.8851	0.0417	0.0000
8/10/00	0.5417	0.0917	0.0000	0.0167	0.5667	0.0083	0.0000
8/24/00	0.0822	0.0519	0.0065	0.0652	0.2058	0.0465	0.0000
9/7/00	0.0278	0.0324	0.0379	0.0866	0.1847	0.1554	0.0000
9/20/00	0.0000	0.0694	0.0000	0.0478	0.1173	0.0556	0.0000
10/3/00	0.0000	0.0368	0.0000	0.0083	0.0451	0.0000	0.0000
Mean	0.2104	0.0473	0.0152	0.0506	0.3159	0.0998	0.0000
5/21/01	0.0833	0.0000	0.0000	0.0000	0.0833	0.8068	0.0000
6/6/01	0.6893	0.0000	0.0000	0.1857	0.8750	0.1250	0.0000
6/18/01	0.0500	0.0000	0.0000	0.0000	0.0500	0.0000	0.0000
7/3/01	0.0343	0.0000	0.0000	0.0000	0.0343	0.0100	0.0000
7/19/01	0.0000	0.1268	0.0000	0.0000	0.1268	0.0250	0.0000
7/30/01	0.0000	0.0000	0.0000	0.0125	0.0125	0.0250	0.0000
8/15/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8/27/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
9/5/01	0.0104	0.0000	0.0000	0.0000	0.0104	0.0625	0.0000
9/18/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.1472	0.0000
Mean	0.0867	0.0127	0.0000	0.0198	0.1192	0.1202	0.0000
5/24/02	0.0000	0.0000	0.0000	0.0000	0.0000	0.0625	0.0000
6/3/02	0.0208	0.0000	0.0000	0.0000	0.0208	0.0046	0.0139
6/17/02	0.0000	0.0196	0.0000	0.0000	0.0196	0.0000	0.0000
7/1/02	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
7/16/02	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
7/29/02	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8/13/02	0.0000	0.0069	0.0000	0.0069	0.0139	0.0228	0.0000
8/26/02	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
9/10/02	0.0000	0.0069	0.0000	0.0139	0.0208	0.0000	0.0000
Mean	0.0023	0.0037	0.0000	0.0023	0.0083	0.0100	0.0015
5/28/03	0.0000	0.0000	0.0000	0.0000	0.0000	0.0208	0.0000
6/11/03	0.0158	0.0000	0.0000	0.0000	0.0158	0.0000	0.0000
6/22/03	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
7/7/03	0.0000	0.0000	0.0000	0.0000	0.0000	0.0069	0.0000
7/24/03	0.0000	0.0000	0.0000	0.0000	0.0000	0.0139	0.0000
8/4/03	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8/20/03	0.0000	0.0000	0.0000	0.0000	0.0000	0.0139	0.0000
Mean	0.0023	0.0000	0.0000	0.0000	0.0023	0.0079	0.0000

Table 9. Continued.

Lake	Date	Eggs	Larvae	Pupae	Adults	Total	Acent	Parap
Auburn								
	5/19/00	0.0267	0.0267	0.0000	0.0000	0.0533	0.0000	0.0000
	6/1/00	0.0000	0.0218	0.0000	0.0079	0.0298	0.0000	0.0000
	6/15/00	0.0139	0.0278	0.0000	0.0000	0.0417	0.0000	0.0000
	6/27/00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	7/10/00	0.0000	0.0000	0.0069	0.0347	0.0417	0.0000	0.0000
	7/25/00	0.1528	0.0000	0.0069	0.0556	0.2153	0.0000	0.0000
	8/9/00	0.0368	0.0515	0.0515	0.0294	0.1691	0.0000	0.0000
	8/28/00	0.0000	0.0000	0.0000	0.0074	0.0074	0.0000	0.0000
	9/12/00	0.0000	0.0208	0.0062	0.0123	0.0394	0.0000	0.0149
	9/28/00	0.0000	0.0000	0.0000	0.0139	0.0139	0.0000	0.0000
	Mean	0.0230	0.0149	0.0072	0.0161	0.0612	0.0000	0.0015
	5/10/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	5/24/01	0.2562	0.0139	0.0000	0.0309	0.3009	0.0000	0.0000
	5/30/01	0.1847	0.0000	0.0000	0.0000	0.1847	0.0000	0.0000
	6/13/01	0.0069	0.0139	0.0139	0.0308	0.0655	0.0000	0.0000
	6/28/01	0.0278	0.0139	0.0000	0.0000	0.0417	0.0000	0.0000
	7/9/01	0.0278	0.1389	0.0139	0.0139	0.1944	0.0000	0.0000
	7/23/01	0.0000	0.0123	0.0270	0.0139	0.0532	0.0000	0.0000
	8/8/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	8/20/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	9/11/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	9/27/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Mean	0.0458	0.0175	0.0050	0.0081	0.0764	0.0000	0.0000
	5/22/02	0.0185	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000
	6/13/02	0.0074	0.0000	0.0000	0.0000	0.0074	0.0000	0.0000
	6/26/02	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	7/11/02	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	7/22/02	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	8/7/02	0.0000	0.0000	0.0000	0.0208	0.0208	0.0000	0.0000
	8/21/02	0.0185	0.0417	0.0024	0.0062	0.0688	0.0000	0.0000
	9/4/02	0.0000	0.0000	0.0000	0.0417	0.0417	0.0000	0.0000
	9/20/02	0.0000	0.0208	0.0417	0.2708	0.3333	0.0000	0.0069
	Mean	0.0049	0.0069	0.0049	0.0377	0.0545	0.0000	0.0008
	5/16/03	0.0820	0.0000	0.0000	0.0093	0.0913	0.0069	0.0000
	5/27/03	0.0324	0.0000	0.0000	0.0069	0.0394	0.0069	0.0000
	6/9/03	0.0079	0.0139	0.0079	0.0000	0.0298	0.0000	0.0000
	6/24/03	0.0000	0.0000	0.0074	0.0221	0.0294	0.0000	0.0000
	7/8/03	0.0000	0.0262	0.0083	0.0179	0.0524	0.0000	0.0000
	7/21/03	0.0780	0.0188	0.0000	0.0000	0.0968	0.0000	0.0000
	8/5/03	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	8/20/03	0.0347	0.0069	0.0000	0.0139	0.0556	0.0000	0.0000
	9/22/03	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Mean	0.0261	0.0073	0.0026	0.0078	0.0439	0.0015	0.0000
Otter								
	6/5/00	0.1940	0.1321	0.0500	0.0821	0.4583	0.0250	0.0000
	6/22/00	0.1395	0.2027	0.0580	0.0804	0.4806	0.0268	0.0089
	7/5/00	0.0000	0.0403	0.0079	0.0079	0.0575	0.0000	0.0000
	7/18/00	0.0000	0.0074	0.0074	0.0000	0.0147	0.0000	0.0000
	8/2/00	0.0218	0.0000	0.0069	0.0218	0.0506	0.0069	0.0000
	8/16/00	0.0074	0.0147	0.0000	0.0000	0.0221	0.0000	0.0000
	8/29/00	0.0000	0.0441	0.0074	0.0515	0.1029	0.0000	0.0000
	9/13/00	0.0000	0.0394	0.0278	0.0231	0.0903	0.0000	0.0000
	9/26/00	0.0000	0.0069	0.0764	0.1042	0.1875	0.0000	0.0000
	Mean	0.0403	0.0542	0.0269	0.0412	0.1627	0.0065	0.0010

Table 9. Continued.

Lake	Date	Eggs	Larvae	Pupae	Adults	Total	Acent	Parap
Otter								
	5/21/01	0.3268	0.0000	0.0000	0.1250	0.4518	0.0000	0.0000
	6/4/01	0.2225	0.0000	0.0000	0.1789	0.4015	0.0417	0.0147
	6/21/01	0.5345	0.0407	0.0000	0.0663	0.6415	0.0074	0.0000
	7/5/01	0.4117	0.1354	0.0851	0.1634	0.7955	0.0202	0.0000
	7/16/01	0.1119	0.0000	0.0000	0.2608	0.3727	0.0000	0.0000
	8/1/01	0.1027	0.0469	0.0000	0.1007	0.2502	0.0000	0.0000
	8/13/01	0.1507	0.0306	0.0000	0.0512	0.2324	0.0000	0.0000
	8/28/01	0.0515	0.1922	0.0000	0.0221	0.2658	0.0074	0.0000
	9/5/01	0.1128	0.1553	0.0131	0.1063	0.3875	0.0378	0.0069
	9/17/01	0.0278	0.2750	0.0486	0.2935	0.6449	0.0069	0.1918
	10/2/01	0.0193	0.0432	0.0288	0.1211	0.2124	0.0455	0.0481
	Mean	0.1884	0.0836	0.0160	0.1354	0.4233	0.0152	0.0238
	5/21/02	0.0179	0.0000	0.0000	0.0625	0.0804	0.0238	0.0000
	6/2/02	0.5218	0.1862	0.0147	0.1183	0.8646	0.0000	0.0715
	6/17/02	0.0981	0.2302	0.0591	0.0757	0.4631	0.0083	0.0000
	7/3/02	0.1759	0.2037	0.0208	0.1319	0.5324	0.0000	0.0069
	7/16/02	0.1911	0.0000	0.0000	0.2444	0.4355	0.0000	0.0069
	7/29/02	0.0294	0.0296	0.0000	0.0795	0.1459	0.0000	0.0131
	8/13/02	0.0964	0.0182	0.0000	0.0339	0.1484	0.0000	0.0000
	8/26/02	0.0672	0.0389	0.0000	0.0546	0.1607	0.0000	0.0000
	9/9/02	0.0208	0.0069	0.0000	0.0208	0.0486	0.0000	0.0000
	Mean	0.1354	0.0793	0.0105	0.0913	0.3200	0.0036	0.0109
	5/21/03	0.2944	0.0062	0.0000	0.0340	0.3345	0.0062	0.0000
	6/5/03	0.2167	0.1379	0.0634	0.0368	0.4622	0.0000	0.0074
	6/18/03	0.0915	0.1612	0.0697	0.0526	0.3253	0.0000	0.0062
	7/3/03	0.1538	0.2083	0.0347	0.0506	0.4474	0.0000	0.0000
	7/15/03	0.0238	0.0300	0.0000	0.0265	0.0406	0.0000	0.0000
	7/29/03	0.0610	0.0866	0.0069	0.0208	0.1754	0.0000	0.0000
	8/14/03	0.0347	0.2083	0.0000	0.0000	0.2431	0.0000	0.0000
	9/19/03	0.0278	0.0208	0.0139	0.0208	0.0833	0.0069	0.0000
	Mean	0.1130	0.1074	0.0236	0.0303	0.2640	0.0016	0.0017
Smith's								
	5/21/99	0.5200	0.0000	0.0000	0.0933	0.6133	-	-
	6/3/99	0.1600	0.0933	0.0000	0.0133	0.2667	-	-
	6/16/99	0.0533	0.1200	0.0000	0.0000	0.1733	-	-
	6/30/99	0.0400	0.0533	0.0000	0.0000	0.0933	-	-
	7/15/99	0.0267	0.1333	0.0000	0.0267	0.1867	-	-
	7/27/99	0.0000	0.1067	0.0133	0.0267	0.1467	-	-
	8/11/99	0.0933	0.3600	0.0000	0.0267	0.4800	-	-
	8/25/99	0.0800	0.5067	0.0133	0.0000	0.6000	-	-
	9/10/99	0.0133	0.2289	0.1333	0.0000	0.3756	-	-
	Mean	0.1096	0.1780	0.0178	0.0207	0.3262		
	5/25/00	0.2867	0.0267	0.0000	0.0000	0.3133	0.0000	0.0000
	6/8/00	0.2095	0.1429	0.0095	0.0000	0.3619	0.0000	0.0000
	6/21/00	0.2519	0.0824	0.0429	0.0167	0.3938	0.0583	0.0000
	7/3/00	0.0810	0.0369	0.0000	0.0000	0.1179	0.0000	0.0000
	7/19/00	0.0167	0.0250	0.0111	0.0417	0.0944	0.0000	0.0000
	8/4/00	0.2604	0.0702	0.1339	0.0274	0.4919	0.0000	0.0000
	8/15/00	0.0472	0.0750	0.0074	0.0389	0.1685	0.0000	0.0000
	8/23/00	0.0919	0.1100	0.0726	0.0871	0.3361	0.0085	0.0000
	9/6/00	0.0250	0.0880	0.0000	0.0591	0.1721	0.0000	0.0000
	9/19/00	0.0000	0.0167	0.0000	0.0167	0.0333	0.0000	0.0000
	Mean	0.1270	0.0674	0.0277	0.0288	0.2483	0.0067	0.0000

Table 9. Continued.

Lake	Date	Eggs	Larvae	Pupae	Adults	Total	Acent	Parap
Smith's								
	5/15/01	0.0000	0.0000	0.0000	0.0083	0.0083	0.0000	0.0000
	5/31/01	0.0241	0.0000	0.0000	0.0333	0.0574	0.0000	0.0000
	6/11/01	0.2287	0.0083	0.0000	0.0095	0.2466	0.0000	0.0000
	6/25/01	0.0222	0.0000	0.0000	0.0274	0.0496	0.0000	0.0000
	7/10/01	0.0000	0.0482	0.0240	0.0000	0.0722	0.0000	0.0000
	7/23/01	0.0000	0.0639	0.0307	0.0000	0.0946	0.0000	0.0000
	8/8/01	0.0250	0.1480	0.0194	0.0083	0.2008	0.0000	0.0000
	8/24/01	0.0148	0.0917	0.0083	0.0000	0.1148	0.0000	0.0000
	9/13/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Mean	0.0350	0.0400	0.0092	0.0096	0.0938	0.0000	0.0000
	6/5/02	0.1790	0.0000	0.0000	0.0079	0.1870	0.0102	0.0000
	6/18/02	0.2113	0.1247	0.0000	0.0000	0.3360	0.0000	0.0000
	7/2/02	0.0676	0.0475	0.0079	0.0119	0.1349	0.0000	0.0000
	7/19/02	0.0111	0.0000	0.0083	0.0194	0.0389	0.0000	0.0000
	8/1/02	0.0167	0.0400	0.0000	0.0328	0.0894	0.0000	0.0000
	8/12/02	0.0000	0.0398	0.0000	0.0083	0.0481	0.0000	0.0000
	8/28/02	0.0083	0.0824	0.0000	0.0324	0.1231	0.0000	0.0000
	9/10/02	0.0000	0.0000	0.0000	0.0102	0.0102	0.0000	0.0000
	Mean	0.0618	0.0418	0.0020	0.0154	0.1210	0.0013	0.0000
	6/3/03	0.0687	0.0077	0.0000	0.0000	0.0764	0.0000	0.0000
	6/18/03	0.1000	0.6446	0.0000	0.0909	0.8355	0.0000	0.0000
	7/1/03	0.0165	0.0165	0.0000	0.0000	0.0330	0.0000	0.0000
	7/16/03	0.0089	0.0170	0.0000	0.0000	0.0259	0.0000	0.0000
	7/31/03	0.0381	0.0116	0.0000	0.0042	0.0539	0.0000	0.0000
	8/12/03	0.0171	0.0313	0.0000	0.0000	0.0484	0.0000	0.0000
	Mean	0.0416	0.1215	0.0000	0.0159	0.1789	0.0000	0.0000

Single surveys (5 transects each) in Cedar and Calhoun during in 2002 and 2003 failed to detect any herbivorous insects in Calhoun and only 0.005 weevils per stem in 2002 (none in 2003) at Cedar. Both lakes have high sunfish densities (>100/trapnet). There was too little milfoil to conduct weevil surveys at Lake of the Isles.

Minneapolis survey lakes:

Milfoil biomass in the four Minneapolis lakes varied among lakes and years (Table 10 and Table 1). Milfoil and total plant biomass was generally low at Lake-of-the-Isles although milfoil biomass exceeded 150 g dry/m^2 in 1996 and 2000. Most of the non-milfoil biomass was coontail. The low densities in most years are likely due to poor water clarity (Table 11); total biomass showed similar patterns, when milfoil was not dominant coontail was the main plant present, and late summer Secchi depths were typically <1.5m. One sample per year does not capture the dynamics of the plants at Isles. For example, just prior to sampling in 2002, milfoil was much more dense (Ward, personal observation), but it declined with a rapid decrease in clarity. Sediment pore water ammonium was moderate (Table 11) and exchangeable N levels were well above those expected for nuisance milfoil (> 0.01 mg N/g sediment).

Table 10. Total plant and milfoil biomass (g dry/m^2) and mean percent of plant biomass that was Eurasian watermilfoil at Minneapolis Chain of Lakes lakes in summer 1999-2003. N ≥ 20 samples at all sites. See Tables 1-3 for Cedar results.

Lake	Date	Total Plant Biomass (g/m^2)	Milfoil Biomass (g/m^2)	% Milfoil (of biomass)	Secchi Depth (m)
Lake of the Isles	9/14/95	62.5	58.3	90.1%	0.5
	SE	20.6	22.6	5.0%	
	8/30/96	199.7	169.2	74.6%	1.1
	SE	74.0	74.1	10.1%	
	8/14/97	31.9	9.9	22.4%	1.4
	SE	10.4	5.3	8.6%	
	8/31/98	28.2	14.0	36.9%	0.3
	1 SE	4.7	6.1	12.2%	
	8/16/99	51.8	49.3	88.3%	0.5
	1SE	14.8	14.5	4.4%	
	6/28/00	265.4	252.9	88.9%	2.3
	1 SE	45.6	46.9	3.7%	
	8/16/00	195.4	192.7	97.7%	2.2
	1 SE	17.6	17.8	1.1%	
	6/27/01	22.0	4.5	30.0%	1.6
	1 SE	7.1	1.8	8.2%	
	9/7/01	16.0	3.0	18.6%	0.8
	1 SE	8.9	2.2	7.9%	
	7/9/02	37.7	24.9	32.4%	1.1
	1 SE	9.4	9.0	9.1%	
	8/22/03	27.3	26.1	79.4%	0.4
	1 SE	18.9	18.5	10.0%	
Calhoun	9/16/99	41.6	8.1	10.8%	1.6
	1 SE	10.7	3.9	5.5%	
	6/26/00	22.7	10.8	38.3%	3.1
	1 SE	11.3	5.6	13.5%	
	8/18/00	12.5	10.9	56.5%	1.8
	1 SE	4.0	4.1	10.0%	
	6/28/01	99.8	98.1	81.0%	3.2
	1 SE	24.9	25.0	7.1%	
	9/6/01	142.1	121.9	73.3%	2.3
	1 SE	30.5	31.3	8.4%	
	7/26/02	181.4	179.5	94.1%	2.8
	1 SE	26.4	26.6	4.3%	
	8/26/03	155.2	154.9	95.9%	2.6
	1 SE	27.1	27.1	3.5%	
Harriet	9/23/99	180.2	168.3	87.9%	2.6
	1 SE	27.6	26.8	5.2%	
	6/30/00	332.1	215.0	61.5%	1.6
	1 SE	53.2	37.8	5.7%	
	8/22/00	106.0	90.7	78.0%	2.3
	1 SE	18.9	19.5	5.9%	
	7/2/01	311.1	259.4	74.1%	2.5
	1 SE	46.4	45.9	6.9%	
	9/12/01	170.5	149.6	83.7%	3.0
	1 SE	25.7	23.6	5.3%	
	7/11/02	252.9	237.3	86.1%	2.2
	1 SE	42.3	44.0	5.0%	
	9/14/02	354.8	337.3	95.5%	2.9
	1 SE	43.6	42.0	1.8%	
	6/16/03	281.9	267.9	91.6%	2.3
	1 SE	46.9	44.3	4.1%	
	8/25/03	252.2	225.0	85.1%	3.3
	1 SE	41.5	40.1	5.3%	

Table 11. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium concentrations) and water column characteristics at Minneapolis Chain of Lakes lakes in summer 1999-2002. Nine sediment samples from the shallow, intermediate and deep stations were collected at each lake.

Lake/Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/m^3)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Plant Limit (m)
Calhoun								
9/24/97				7.2	3.1	18.9	2.5-3.0	4.7
9/4/98				3.7	3.0	23.7	3.5-4.0	4.1
9/21/99				17.1	1.6	18.5	2.0	3.8
6/26/00	0.75	2.00	6.17	4.3	3.1	21.4	3.5-4	
2se	0.32	1.08	2.60					
8/18/00	0.65	1.15	0.17	8.6	1.8	24.3	3.5-4	2.4
2se	0.38	0.33	0.03					
6/28/01	0.68	1.31	6.0	19.8	3.2	26.1	3.5	
2se	0.31	1.02	2.4					
9/6/01	0.68	2.96	7.6	3.5	2.3	22.9	5	4.8
2se	0.40	1.58	3.2					
7/26/02	0.74	6.62	15.3	.	2.8	25.2	3.5	
2se	0.37	4.33	14.3					
8/23/02	.	.	.	11.2	2.2	22.1	3-3.5	5.1
2se	.	.	.					
8/5/03	0.61	2.69	6.1	.	2.6	25.5	4	4.5
2se	0.27	1.37	2.4					
Lake of the Isles								
9/14/95	1.45	5.21	1.8	57.4	0.5	20.3	0.5-1.0	0.5
2se	0.36	4.36	1.1					
8/30/96	0.28	9.30	10.0	6.9	1.1	24.6	1.5-2.0	2.0
2se	0.08	5.32	6.7					
8/13/97	0.71	8.48	16.2	26.2	1.4	22.5	1.0-1.5	3.7
2se	0.58	0.88	20.0					
8/31/98	0.25	29.33	23.9	54.3	0.3	24.3	0.5-1.0	3.3
2se	0.28	19.07	19.0					
8/16/99	0.15	0.54	24.2	83.7	0.5	22.5	0.5-1.0	3.0
2se	0.05	0.56	12.5					
6/28/00	0.72	0.57	41.1	8.8	2.3	22.9	1.5-2.0	
2se	0.87	0.23	13.3					
8/16/00	0.51	1.13	26.1	15.8	2.2	25.7	2.5-3.0	4.0
2se	0.39	1.09	12.8					
6/29/01	0.95	2.55	16.8	49.5	1.6	26.3	2.0-2.5	
2se	0.49	1.96	14.1					
9/7/01	0.53	3.42	27.6	42.8	0.8	23.5	1.0-1.5	2.6
2se	0.44	1.38	15.8					
7/9/02	0.60	2.66	42.1	.	1.1	28.4	1.0-1.5	
2se	0.66	2.03	55.7					
8/22/02				82.3	0.7	22.7	1	3.9
8/5/03	0.69	3.74	22.7	.	0.4	25.5	0.5-1.0	3.7
2se	0.44	1.46	16.0					

Table 11. continued

Lake/Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/m^3)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Plant Limit (m)
Harriet								
10/9/97				4.5	> 5.4	17.3	3.0-3.5	5.2
9/23/98				3.7	2.6	20.3	4.0-4.5	5.0
9/24/99				7.5	2.6	17.5	3.5	4.0
6/30/00	0.74	3.74	7.69	6.1	1.6	22.8	2.5-3	
2se	0.42	1.43	3.87					
8/22/00	0.76	6.72	.	8.3	2.3	23.1	3.5-4	4.2
2se	0.48	1.59	.					
7/2/01	0.94	3.59	7.0	9.1	2.5	23.4	2.5-3.0	
2se	0.44	2.31	3.6					
9/12/01	0.78	2.13	7.3	4.0	3.6	21.5	4.5-5.0	4.3
2se	0.44	1.21	3.7					
7/11/02	1.23	3.28	6.1	7.4	2.2	25.4	3.5	
2se	0.44	1.64	1.1					
9/14/02	.	.	.	.	2.9	23.1	4.0	4.2
8/25/03	0.44	3.62	10.8	.	2.3	26.4	4	4.9
2se	0.32	1.07	3.7					
9/4/03				.	3.3	22.9	4	
2se								

Milfoil biomass increased at Lake Calhoun from very low levels in 1999-2000 (Table 10) to 150-180 g/m^2 in 2002 and 2003 when it composed > 94% of total plant biomass. It is unclear why biomass was low in 1999-2000, but biomass of all plants was low both years. Sediment characteristics and clarity were not notably different from the more recent years with higher density (Table 11). Exchangeable N was well above levels for nuisance milfoil in June 2001 and almost as high in 2002. Unfortunately detailed sediment data are not available 1999 and exchangeable N was not measured in 2000. Milfoil biomass was quite high at the connected Lake-of-the-Isle in 2000 so the low biomass in 2000 must be related to Calhoun specific conditions.

Milfoil biomass has been consistently high at Lake Harriet ranging from 170 g/m^2 in 1999 to over 325 g/m^2 in 2002 (Table 10). Milfoil typically composed 85-95% of total plant biomass at Harriet. Water clarity was similar to Calhoun as were sediment characteristics (Table 11), however milfoil and total plants were much more abundant at Harriet in 1999 and 2000 than they were at Calhoun and in subsequent years, plants were twice as dense at Harriet than at Calhoun. Harriet biomass was more similar to Cedar Lake (Table 1) with milfoil dominating, followed by coontail.

Plant coverage and occurrence (Table 12) showed trends similar to biomass. At Cedar Lake, milfoil occurred at 80-90% of sample locations and was visible at 66-80% of stations. Density was lowest (2.8) in 2001 when biomass was lowest. Coontail was generally the second most frequent and dense plant, occurring at 25-50% of stations. More species are found in the whole lake surveys than in biomass samples, but rarely more than 6 species were found at Cedar Lake. Weevil damage was extremely rare.

Whole lake estimates at Lake Calhoun reflect the low biomass found in 1999-2000 and indicate a decline from levels in 1998, with an increase from 2001-2003. Milfoil density dropped from 3.7 in 1998 to 1.8 and 1.6 in 1999 and 2000 respectively before increasing to 3.7 in 2003. Coontail was the second most common plant at Calhoun but the number of species was higher than Cedar and Isles. Typically 6-12 species were found at Calhoun, although with the exception of coontail, they were infrequent and had density ratings <0.5. The number of species found decreased in 2002 and 2003 as milfoil returned to dominance. Very little weevil damage was noted.

Milfoil coverage and occurrence was consistently high at Lake Harriet. Milfoil occurred at 75 to 85% of station and density ranged from 3.4 to 4.4. Coontail was also more frequent and dense than at the other lakes generally occurring at more than half the sites with a density rating of 2 to 3. Typically 5-7 species were found but the total number of species collected declined in 2002-2003 (Table 12). Species other than milfoil and coontail were infrequent and at low density. Weevil damage was also low at Harriet.

Lake-of-the-Isles showed the greatest variation in coverage and density. In several years coontail was more frequent or denser than milfoil. Density and coverage were highest in 2000 when biomass was high and density generally followed biomass trends but did not fluctuate as much as biomass. Coverage and density were much lower in 2001-2003 than in 2000, probably due to poorer clarity (Table 11). Typically 4-6 species were found in Lake of the Isles and the low number of species appears to be as much related to water clarity as it is to milfoil density. Weevil damage was also rare at Lake of the Isles.

It should be noted that we expected that alum treatments in the Minneapolis Chain-of-Lakes would eventually enhance native plant communities. Although we predicted that Eurasian watermilfoil would initially be enhanced by better water clarity, we expected that better water clarity would favor the native plants after several years, reducing the competitive advantage Eurasian watermilfoil appears to have in lower light environments. To date we have no indication that alum treatments have enhanced the native plant communities. Eurasian watermilfoil remained dominant in Cedar Lake, 7 years after treatment in 1996. The number of plant species remains low and the better clarity appears to have reduced seasonal fluctuations in milfoil biomass. Eurasian watermilfoil increased and also remains dominant in Harriet and Calhoun, although the alum treatments are likely too recent to have resulted in a longer-term shift in plant community composition. However, it should also be noted that there are few milfoil weevils in any of these lakes and a shift to native communities may not occur without some additional factor, such as herbivory, limiting Eurasian watermilfoil.

Coverage and density of milfoil was generally lower at the three additional lakes surveyed in 2002, Centerville, Schultz and Vadnais (Table 12), but relative densities were moderate (2.5-3.25). Coontail was the dominant native plant in these lakes. Poor clarity and high chlorophyll (Table 13) probably limited coverage and plant growth in these lakes, although weevils (see below) may also be a factor.

Table 12. Estimates of plant coverage and occurrence for the whole-lake surveys (Calhoun, Cedar, Harriet, Isles, Centerville, Schultz and Vadnais). Estimates of visual milfoil cover (% Vis MSP Cov), percent visual occurrence, occurrence on the drop hook and mean weevil damage rating (0-5) for the whole lake estimates were based on n = 66-82 stations at each of the Minneapolis lakes and 25-30 stations at Centerville, Schultz and Vadnais. Jessen and Lound (1962) relative density ratings (0-5) were determined from a subset of 5-6 transects (n=24-29 stations). Relative density is the mean for all stations sampled. Species abbreviations are given in Appendix I.

Cedar Lake		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 26
Date	n	Mean ± 1S.E.	Spp.% Occ. ± 1S.D.	Spp.% Occ. ± 1S.D.	Spp.Density ± 2S.E.
9/27/99	75	50.1 ± 4.2%	MSP 78.7 ± 4.7%	MSP 90.7 ± 3.4%	MSP 3.96 ± 0.46
			NMP 13.3 ± 3.9%	CRT 25.3 ± 5.0%	CRT 1.50 ± 0.60
				NMP 6.7 ± 2.9%	NMP 0.12 ± 0.23
					PRI 0.04 ± 0.08
					DRC 0.04 ± 0.08

Cedar Lake		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 24
Date	n	Mean ± 1S.E.	Spp.% Occ. ± 1S.D.	Spp.% Occ. ± 1S.D.	Spp.Density ± 2S.E.
8/9/00	72	44.3 ±4.7%	MSP 68.1 ± 5.5%	MSP 87.5 ± 3.9%	MSP 3.58 ± 0.61
			CRT 9.7 ± 3.5%	CRT 23.6 ± 5.0%	CRT 1.29 ± 0.53
Eurasian Watermilfoil			NMP 15.3 ± 4.2%	NAJ 1.4 ± 1.4%	NMP 0.38 ± 0.38
Total Area: 17.7 ha.			PAM 1.4 ± 1.4%	NMP 6.9 ± 3.0%	NAJ 0.08 ± 0.17
% of Litt. Zone: 69.4%			PEC 1.4 ± 1.4%	PAM 1.4 ± 1.4%	CHA 0.04 ± 0.08
% of Lake Area: 26.7%				PCR 1.4 ± 1.4%	
				CHA 1.4 ± 1.4%	

Cedar Lake		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 24
Date	n	Mean 1S.E.	Spp.% Occ. 1S.D.	Spp.% Occ. 1S.D.	Spp.Density 2S.E.
8/21/01	75	36.3 ± 4.2%	MSP 66.7 ± 5.4%	MSP 81.3 ± 4.5%	MSP 2.83 ± 0.71
			NMP 16.0 ± 4.2%	CRT 34.7 ± 5.5%	CRT 0.71 ± 0.52
			CRT 9.3 ± 3.4%	NMP 5.3 ± 2.6%	NMP 0.08 ± 0.17
			PEC 1.3 ± 1.3%	CHA 1.3 ± 1.3%	
			PRI 1.3 ± 1.3%	PEC 1.3 ± 1.3%	
			PZS 1.3 ± 1.3%	PRI 1.3 ± 1.3%	
Weevil Damage Rating:		0.24			

Cedar		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n =18
Date	n	Mean ± 1SE	Spp. % Occ. ± 1 SD	Spp. % Occ. ± 1 SD	Spp. Density ± 2SE
8/26/02	68	56.6% ±4.6%	MSP 77.9 ± 0.1	MSP 83.6 ± 0.0	MSP 4.44 ± 0.29
			CRT 19.1 ± 0.0	CRT 47.1 ± 0.1	CRT 2.00 ± 0.76
Eurasian Watermilfoil			PAM 5.9 ± 0.0	PAM 4.4 ± 0.0	PAM 0.28 ± 0.56
Total Area:		21.6 ha.	NMP 4.4 ± 0.0	PPR 4.4 ± 0.0	
% of Litt. Zone:		84.6%	PPR 4.4 ± 0.0	NMP 2.9 ± 0.0	
% of Lake Area:		32.5%	PCR 1.5 ± 0.0		
Weevil Damage Rating:		0.31			

Cedar		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n =26
Date	n	Mean ± 1SE	Spp. % Occ. ± 1 SD	Spp. % Occ. ± 1 SD	Spp. Density ± 2SE
8/18/03	74	34.7% ±4.4%	MSP 66.2 ± 0.1	MSP 83.8 ± 0.0	MSP 3.2 ± 0.7
			CRT 21.6 ± 0.0	CRT 47.3 ± 0.1	CRT 1.8 ± 0.6
			NMP 17.6 ± 0.0	NMP 8.1 ± 0.0	NMP 0.2 ± 0.3
			PGR 2.7 ± 0.0	PGR 2.7 ± 0.0	PRI 0.1 ± 0.2
Weevil Damage Rating:		0.25		PRI 1.4 ± 0.0	PGR 0.2 ± 0.3

Table 12 Continued

Lake Calhoun		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 27
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
9/4/98	63	30.7 ± 4.4%	MSP	87.3 ± 4.2%	MSP	76.2 ± 5.4%	MSP	3.67 ± 0.49
			PEC	17.5 ± 4.8%	CRT	50.8 ± 6.3%	CRT	3.07 ± 0.53
Eurasian Watermilfoil			PRI	14.3 ± 4.4%	PEC	12.7 ± 4.2%	PCR	0.48 ± 0.38
Total Area: 27.9 ha.			CRT	11.1 ± 4.0%	PRI	3.2 ± 2.2%	PEC	0.48 ± 0.43
% of Litt. Zone: 56%			PCR	7.9 ± 3.1%	PZS	1.6 ± 1.6%	PRI	0.41 ± 0.36
% of Lake Area: 16.7%			NAJ	6.3 ± 3.1%			NAJ	0.33 ± 0.34
			ELD	1.6 ± 1.6%			ELD	0.04 ± 0.07
Weevil Damage Rating: 0.698±0.133			HET	1.6 ± 1.6%			HET	0.04 ± 0.07

Lake Calhoun		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 25
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE.
9/16/99	74	45.0± 4.5%	MSP	87.3 ± 3.9%	MSP	76.2 ± 5.0%	MSP	1.84 ± 0.75
			PEC	17.5 ± 4.4%	CRT	50.8 ± 5.8%	CRT	3.32 ± 0.47
Eurasian Watermilfoil			PRI	14.3 ± 4.1%	PEC	12.7 ± 3.9%	PRI	0.20 ± 0.23
Total Area:			CRT	11.1 ± 3.7%	PRI	3.2 ± 2.0%		
% of Litt. Zone:			PCR	7.9 ± 3.1%	PZS	1.6 ± 1.5%		
% of Lake Area:			NAJ	6.3 ± 2.8%				
			ELD	1.6 ± 1.5%				
Weevil Damage Rating:			HET	1.6 ± 1.5%				

Lake Calhoun		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook		Density Rating	n = 26
Date	n	Mean ±1S.E.	Spp.	% Occ. ±1S.D.	Spp.	% Occ. ±1S.D.	Spp.	Density ±2S.E.
8/17/00	73	6.8±2.0%	MSP	26.0 ± 5.1%	MSP	24.7 ± 5.0%	MSP	1.62 ± 0.70
			PEC	1.4 ± 1.4%	CRT	11.0 ± 3.7%	PEC	0.04 ± 0.08
Eurasian Watermilfoil			PRI	2.7 ± 1.9%	NAJ	2.7 ± 1.9%	PZS	0.12 ± 0.17
Total Area: 10.4 ha.			NAJ	1.4 ± 1.4%	PRI	2.7 ± 1.9%	CRT	2.00 ± 0.63
% of Litt. Zone: 20.9%			CHA	1.4 ± 1.4%	PZS	1.4 ± 1.4%	ELD	0.04 ± 0.08
% of Lake Area: 6.2%							PCR	0.38 ± 0.35
							NAJ	0.31 ± 0.29
							PRI	0.12 ± 0.17
							HET	0.08 ± 0.15
							CHA	0.42 ± 0.32
							VAL	0.04 ± 0.08
							ZPA	0.15 ± 0.31

Lake Calhoun		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook		Density Rating	n = 26
Date	n	Mean ±1S.E.	Spp.	% Occ. ±1S.D.	Spp.	% Occ. ±1S.D.	Spp.	Density ±2S.E.
8/17/01	66	31.3 ± 4.9%	MSP	39.4 ± 6.0%	MSP	56.1 ± 6.1%	MSP	2.62 ± 0.62
			PEC	7.6 ± 3.3%	CRT	15.2 ± 4.4%	NAJ	0.54 ± 0.40
Eurasian Watermilfoil			CRT	3.0 ± 2.1%	PEC	7.6 ± 3.3%	CRT	0.46 ± 0.28
Total Area: 31.5 ha.			PCR	3.0 ± 2.1%	PRI	6.1 ± 2.9%	PRI	0.27 ± 0.38
% of Litt. Zone: 63.2%			NAJ	1.5 ± 1.5%	NAJ	3.0 ± 2.1%	PCR	0.19 ± 0.19
% of Lake Area:18.8%			PZS	1.5 ± 1.5%	PZS	3.0 ± 2.1%	PEC	0.15 ± 0.24
					PCR	1.5 ± 1.5%	PZS	0.15 ± 0.24
Weevil Damage Rating:		0.2			PFO	1.5 ± 1.5%	PPR	0.12 ± 0.23
							CHA	0.08 ± 0.11
							HET	0.04 ± 0.08
							PFO	0.04 ± 0.08

Calhoun		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n =25
Date	n	Mean ±1S.E.	Spp.	% Occ. ±1S.D.	Spp.	% Occ. ±1S.D.	Spp.	Density ±2S.E.
8/20/02	68	52.2 ± 4.0%	MSP	80.9 ± 0.0	MSP	71.4 ± 0.1	MSP	3.16 ± 0.71
			CRT	7.5 ± 0.0	CRT	19.0 ± 0.0	CRT	0.16 ± 0.19
			PRI	6.9 ± 0.0	PRI	4.8 ± 0.0	NAJ	0.04 ± 0.08
			VAL	2.9 ± 0.0	NAJ	1.6 ± 0.0	PRI	0.28 ± 0.29
			PEC	1.5 ± 0.0			VAL	0.04 ± 0.08
Weevil Damage Rating:		0.15	PIL	1.5 ± 0.0				

Table 12 Continued

Calhoun		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n =27
Date	n	Mean ±1S.E.	Spp.	% Occ. ±1S.D.	Spp.	% Occ. ±1S.D.	Spp.	Density ±2S.E.
8/13/03	74	34.8% ±4.0%	MSP	63.5 ± 0.1	MSP	85.1 ± 0.0	MSP	3.7 ± 0.4
			CRT	2.7 ± 0.0	CRT	5.4 ± 0.0	CRT	0.4 ± 0.3
			PEC	2.7 ± 0.0	PEC	1.4 ± 0.0	PEC	0.1 ± 0.1
			NAJ	1.4 ± 0.0	PRI	1.4 ± 0.0	NAJ	0.2 ± 0.3
							PRI	0.0 ± 0.1
Weevil Damage Rating: 0.61							CHC	0.2 ± 0.3

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 29
Date	n	Mean ± 1 S.E.	Spp.	% Occ. ± 1 S.D.	Spp.	% Occ. ± 1 S.D.	Spp.	Density ± 2S.E.
10/9/97	72	52.2 ± 3.8%	MSP	87.5 ± 3.9%	MSP	86.1 ± 4.1%	MSP	4.41 ± 0.36
Eurasian Watermilfoil:			CRT	8.3 ± 3.3%	CRT	40.3 ± 5.8%	CRT	2.21 ± 0.49
Total Area:		31.4 ha.	HET	1.4 ± 1.4%	PRI	1.4 ± 1.4%	PRI	0.17 ± 0.14
% of Litt. Zone:		91.2%	PRI	1.4 ± 1.4%	PZS	1.4 ± 1.4%	ELD	0.03 ± 0.07
% of Lake Area:		22.7%					NAJ	0.03 ± 0.07
							PEC	0.03 0.07

Weevil Damage rating 0.507±0.072

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 27
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
9/23/98	73	59.2 ± 4.2%	MSP	84.9 ± 4.2%	MSP	82. ± 4.5%	MSP	3.81 ± 0.68
			CRT	8.2 ± 3.2%	CRT	39.7 ± 5.7%	CRT	2.07 ± 0.55
Eurasian Watermilfoil:			PRI	6.8 ± 3.0%	PRI	6.8 ± 3.0%	PRI	0.26 ± 0.31
Total Area:		25.9 ha.	NAJ	1.4 ± 1.4%	NAJ	5.7 ± 2.7%	PZS	0.19 ± 0.26
% of Litt. Zone:		75.3%	PZS	1.4 ± 1.4%	PEC	1.4 ± 1.4%	NAJ	0.15 ± 0.18
% of Lake Area:		18.7%			PZS	1.4 ± 1.4%	PEC	0.07 ± 0.10
							HET	0.04 ± 0.07

Weevil Damage Rating: 0.493±0.088

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 29
Date	n	Mean ±1S.E.	Spp.	% Occ. ± 1S.D.	Spp. ±	% Occ. ±S.D.	Spp.	Density ±2S.E.
9/24/99	71	71.9 ±2.8%	MSP	79.2 ± 4.8%	MSP	93.1 ± 3.0%	MSP	3.86 ± 0.44
			CRT	11.1 ± 3.7%	CRT	59.7 ± 5.8%	PZS	0.03 ± 0.07
							CRT	3.14 ± 0.46

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 25
Date	n	Mean ±1S.E.	Spp.	% Occ. ±1S.D.	Spp.	% Occ. ±1S.D.	Spp.	Density ±2S.E.
8/21/00	66	36.8 ±4.2%	MSP	71.2 ± 5.6%	MSP	74.2 ± 5.4%	MSP	3.56 ± 0.54
			CRT	24.2 ± 5.3%	CRT	62.1 ± 6.0%	PEC	0.12 ± 0.13
Eurasian Watermilfoil:			NAJ	1.5 ± 1.5%	NAJ	1.5 ± 1.5%	PZS	0.08 ± 0.16
Total Area:		21.1 ha.	PZS	3.0 ± 2.1%	PZS	1.5 ± 1.5%	CRT	3.20 ± 0.60
% of Litt. Zone:		61.3%	PEC	3.0 ± 2.1%			NAJ	0.12 ± 0.24
% of Lake Area:		15.3%					PRI	0.04 ± 0.08
							CHA	0.04 ± 0.08

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 20
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
8/14/01	71	46.4 ±4.7%	MSP	54.9 ± 5.9%	MSP	81.7 ± 4.6%	MSP	3.65 ± 0.55
			CRT	14.1 ± 4.1%	CRT	60.6 ± 5.8%	CRT	3.05 ± 0.59
			HET	1.4 ± 1.4%	PRI	1.4 ± 1.4%	HET	0.10 ± 0.14
			PEC	1.4 ± 1.4%			NAJ	0.05 ± 0.10
							PRI	0.05 ± 0.10
Weevil Damage Rating:		0.01					PZS	0.05 ± 0.10

Table 12 Continued

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 20
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
8/19/02	n=66	62.1 ±4.6%	MSP	83.3 ± 0.0	MSP	75.8 ± 0.1	MSP	3.40 ± 0.70
			CRT	10.6 ± 0.0	CRT	34.8 ± 0.1	CRT	2.15 ± 0.71
Weevil Damage Rating: 0.36							PRI	0.05 ± 0.10

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 27
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
9/4/03	n=74	48.9 ± 4.5%	MSP	77.0 ± 0.0	MSP	85.1 ± 0.0	MSP	3.6 ± 0.5
			CRT	5.4 ± 0.0	CRT	59.5 ± 0.1	CRT	2.9 ± 0.5
			PEC	2.7 ± 0.0	PCR	1.4 ± 0.0	PEC	0.2 ± 0.2
Weevil Damage Rating: 0.54					PEC	1.4 ± 0.0		

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 25
Date	n	Mean ± 1 S.E.	Spp.	% Occ. ± 1 S.D.	Spp.	% Occ. ± 1 S.D.	Spp.	Density ± 2S.E.
8/13/97	72	15.4 ± 3.5%	MSP	31.9 ± 5.5%	MSP	59.7 ± 5.8%	CRT	2.48 ± 0.37
Eurasian Watermilfoil:			CRT	26.4 ± 5.2%	CRT	62.5 ± 5.7%	MSP	1.84 ± 0.53
Total Area:		13.9 ha.	PZS	1.4 ± 1.4%	NAJ	2.8 ± 1.9%	PZS	0.04 ± 0.08
% of Litt. Zone:		38.5%			PZS	2.8 ± 1.9%		
% of Lake Area:		31.8%						

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 26
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
8/31/98	73	8.5 ± 2.0%	MSP	28.8 ± 5.3%	MSP	56.2 ± 5.8%	CRT	2.85 ± 0.60
Eurasian Watermilfoil			CRT	15.1 ± 4.2%	CRT	39.7 ± 5.7%	MSP	2.81 ± 0.69
Total Area:		36.0 ha.			CHC	2.7 ± 1.9%	NAJ	0.08 ± 0.15
% of Litt. Zone:		100.0%			NAJ	2.7 ± 1.9%	CHC	0.04 ± 0.08
% of Lake Area:		49.6%			PEC	1.4 ± 1.4%	PCR	0.04 ± 0.08
Weevil Damage Rating: 1.411±0.320							PEC	0.04 ± 0.08

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 26
Date	n	Mean ±1S.E.	Spp.	% Occ. ± 1S.D.	Spp.	% Occ. ± 1S.D.	Spp.	Density ± 2S.E.
8/17/99	72	21.2 ± 2.8%	MSP	22.2 ± 4.9%	MSP	72.2 ± 5.3%	MSP	3.69 ± 0.57
			CRT	1.4 ± 1.4%	CRT	40.3 ± 5.8%	PEC	0.04 ± 0.08
							CRT	2.88 ± 0.52
							NAJ	0.04 ± 0.08
							CHA	0.04 ± 0.08

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 26
Date	n	Mean ±1S.E.	Spp.	% Occ. ± 1S.D.	Spp.	% Occ. ± 1S.D.	Spp.	Density ± 2S.E.
8/14/00	82	50.7 ± 4.4%	MSP	82.2 ±14.2%	MSP	87.7 ±13.6%	MSP	3.73 ± 0.49
Eurasian Watermilfoil					CRT	24.7 ±14.8%	CRT	1.58 ± 0.58
Total Area:							PCR	0.23 ± 0.26
% of Litt. Zone:							NAJ	0.04 ± 0.08
% of Lake Area:							PRI	0.04 ± 0.08

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 26
Date	n	Mean ±1S.E.	Spp.	% Occ. ± 1S.D.	Spp.	% Occ. ± 1S.D.	Spp.	Density ± 2S.E.
8/15/01	82	3.9 ± 1.4%	MSP	7.3 ± 2.9%	MSP	25.6 ± 4.8%	CRT	2.88 ± 0.56
			CRT	7.3 ± 2.9%	CRT	36.6 ± 5.3%	MSP	1.65 ± 0.68
Eurasian Watermilfoil					NAJ	1.2 ± 1.2%	NAJ	0.08 ± 0.15
Total Area:		5.4 ha.			PCR	1.2 ± 1.2%	PCR	0.08 ± 0.15
% of Litt. Zone:		15.1%					PFO	0.04 ± 0.08
% of Lake Area:		12.5%					PRI	0.04 ± 0.08
Weevil Damage Rating:		0.15						

Table 12 Continued

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 21
Date	n	Mean ±1S.E.	Spp.% Occ. ± 1S.D.	Spp.% Occ. ± 1S.D.	Spp.Density ± 2S.E.
8/22/02	70	17.3 ± 3.6%	MSP 39.0 ± 0.1	MSP 55.7 ± 0.1	MSP 2.90 ± 0.79
Eurasian Watermilfoil			CRT 19.5 ± 0.0	CRT 40.0 ± 0.1	CRT 1.67 ± 0.68
Total Area:		12.7 ha.	BRA 1.2 ± 0.0	CHA 1.4 ± 0.0	CHA 0.05 ± 0.10
% of Litt. Zone:		35.3%	PEC 1.2 ± 0.0		
% of Lake Area:		29.1%			
Weevil Damage Rating: 0.06					

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 27
Date	n	Mean ±1S.E.	Spp.% Occ. ± 1S.D.	Spp.% Occ. ± 1S.D.	Spp.Density ± 2S.E.
8/6/03	74	4.2% ±.3%	MSP ± 18.9 0.0	MSP 48.6 ± 0.1	MSP 1.5 ± 0.6
			CRT ± 12.2 0.0	CRT 23.0 ± 0.0	CRT 1.2 ± 0.6
Weevil Damage Rating: 0.28					PRI 0.0 ± 0.1

Centerville		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 20
Date	n	Mean 1S.E.	Spp.% Occ. ± 1S.D.	Spp.% Occ ±. 1S.D.	Spp.Density ± 2S.E.
8/14/02	35	0.3 ±0.2%	MSP 8.6 ± 0.0	MSP 71.4 ± 0.1	MSP 3.25 ± 0.66
			CRT 2.9 ± 0.0	CRT 71.4 ± 0.1	CRT 1.65 ± 0.57
			LTR 2.9 ± 0.0	CHA 22.9 ± 0.1	PCR 0.05 ± 0.10
			PEC 2.9 ± 0.0	PCR 2.9 ± 0.0	CHA 0.80 ± 0.64
Weevil Damage Rating:		0.79		PEC 2.9 ± 0.0	

Schultz		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n =24
Date	n	Mean 1S.E.	Spp.% Occ. ± 1S.D.	Spp.% Occ. ± 1S.D.	Spp.Density ± 2S.E.
9/3/02	25	16.6 ±4.4%	MSP 80.8 ± 0.1	MSP 84.6 ± 0.1	MSP 2.46 ± 0.58
			CRT 69.2 ± 0.1	CRT 100.0 ± 0.0	PEC 0.04 ± 0.08
			PEC 30.8 ± 0.1	PAM 30.8 ± 0.1	CRT 3.38 ± 0.66
			PAM 23.1 ± 0.1	PEC 19.2 ± 0.1	PAM 0.83 ± 0.62
			NAJ 3.8 ± 0.0	NAJ 7.7 ± 0.1	
				PCR 7.7 ± 0.1	

Vadnais		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n =31
Date	n	Mean 1S.E.	Spp.% Occ. ± 1S.D.	Spp.% Occ. ± 1S.D.	Spp.Density ± 2S.E.
8/16/02	34	22.4 ±3.8%	MSP 55.9 ± 0.1	MSP 82.4 ± 0.1	MSP 2.65 ± 0.48
			CRT 38.2 ± 0.1	CRT 82.4 ± 0.1	PEC 0.58 ± 0.40
			PEC 26.5 ± 0.1	PEC 38.2 ± 0.1	PZS 0.90 ± 0.40
			VAL 23.5 ± 0.1	VAL 35.3 ± 0.1	CRT 2.97 ± 0.51
			PRI 11.8 ± 0.1	PZS 23.5 ± 0.1	NMP 0.03 ± 0.06
			PZS 8.8 ± 0.0	PPR 20.6 ± 0.1	NAJ 0.10 ± 0.19
			PPR 5.9 ± 0.0	PRI 5.9 ± 0.0	PRI 0.10 ± 0.14
			NAJ 2.9 ± 0.0	NAJ 2.9 ± 0.0	PPR 0.29 ± 0.19
Weevil Damage Rating: 0.49			NMP 2.9 ± 0.0	NMP 2.9 ± 0.0	VAL 0.87 ± 0.55

Table 13. Water column characteristics at additional survey lakes in summer 2002 and sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium concentrations) at a subset of these lakes.

Lake/Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/m^3)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)
Bald Eagle							
8/5/02				53.4	0.8	24.7	0.5-1.0
Centerville							
8/14/02	1.00	10.20	13.5	39.0	1.1	25.9	1.5
2se	0.61	.	7.4				
Independence							
7/31/02				38.2	1.0	26.5	1.0-1.5
Peltier							
7/30/02				85.3	0.8	25.1	1.0
Schultz							
9/3/02				20.0	2.0	24.4	2.0
Vadnais							
8/7/02	1.40	1.24	7.5	15.2	1.7	23.5	2
2se	0.23	.	5.8				

Surveys of weevils and fish

To attempt to detect additional declines and to determine if agent and perhaps milfoil density may be related to fish density, we also conducted weevil surveys on 6 new lakes along with Cedar Lake and Calhoun in August 2002. These lakes had DNR fish surveys conducted in 2000, 2001 or 2002 (Table 14). A range of weevil densities was found; generally lakes with high fish densities had low weevil densities and lakes with high weevil densities had low sunfish densities (Table 14). There was a significant ($p = 0.05$) regression of adult weevil density on ln(sunfish/trapnet):

$$\text{Adults/stem} = 0.16 - 0.034 \ln(\text{sunfish/trapnet}),\ r^2 = 0.49$$

Abundance of sunfish that results in zero weevils can be predicted from the converse regression, which gave an intercept of 4.36, or 78 sunfish per trapnet. The regression of sunfish on total weevil abundance was marginally significant ($p=0.1$).

To increase sample size we included lakes for which fisheries surveys were available and for which we had weevil surveys during the same year. For Cenaiko Lake in 1998 we had one weevil survey from September, one week prior to the fisheries survey. For Lake Auburn in 2000, Cenaiko in 2002 and Otter Lake in 2001 and 2002 we averaged our bi-weekly weevil surveys to provide an average summer density. We then used the combined data set to determine the relationship between weevil density and sunfish relative abundance (Fig. 7). Cenaiko Lake in 1998 was determined to be an outlier (weevil density was much higher than all other sites, Table 14) and was dropped from the regressions (Fig. 7). Because the relationship with total weevil density appeared bimodal, we used a logistic regression for total weevil density, using a threshold of <0.2 weevils/stem (low) or >0.2 weevils/stem (high). The regressions of total weevil density and adult weevil density on ln(sunfish/trapnet) were highly significant (p=0.003 and p=0.001, respectively).

For adult weevils (Fig 7):
Adult weevils per stem = 0.146 - 0.071 $\log_{10}$[sunfish/trapnet], $r^2 = 0.71$

Thus sunfish catch rates explain 70% of the variation in adult weevil abundance across the lakes. Because sunfish prey directly on adult weevils (Sutter and Newman 1997) a direct relationship with adult density makes sense.

With total weevil density (sum of eggs, larvae, pupae and adults), the relationship is clearly bimodal with high and low weevil densities. Because sunfish do not prey on eggs and pupae and larvae are relatively immune to predation the indirect effects of predation on adults might be expected to result in a threshold with low predation allowing high densities and higher predation inhibiting development of significant weevil populations.

The logistic regression of qualitative (high vs. low) total weevil density on sunfish catch rate was highly significant (G^2=8.77, P=0.003) and explained 57% of the variation in qualitative total weevil density. The logistic model suggests a threshold catch rate of 30 sunfish per trap net, above which weevil populations will be at low density (<0.1/stem, Fig. 7).

These regressions suggest that sunfish density explains 60 and 70% of the variation in total weevil and adult weevil density, respectively, among lakes and support our experimental observations that sunfish predation is an important factor limiting weevil density (and thus milfoil control) in Minnesota lakes. The stronger relationship between sunfish and adult densities is intuitively appealing as sunfish prey primarily on adults (Sutter and Newman 1997) and thus indirectly limit total weevil densities. The high density of weevils in Cenaiko in 1998 is consistent with the other results and suggests that at some low fish density, fish are not limiting weevil populations; modeling suggests that with low adult mortality, fall densities can be very high (Ward 2002). The regressions suggest that weevil populations would be below detection with about 80 sunfish per trapnet. A density of more than 25-30 sunfish per trapnet would result in weevil densities less than 0.1/stem and likely be limiting to milfoil control.

There was no clear relationship between weevil density and milfoil relative density at the survey lakes (Tables 12 and 14), however, without several years of data it is difficult to tell if weevil densities had recently increased or if milfoil density was increasing or decreasing.

Table. 14. Results of mid-summer 2002 weevil surveys (number per stem) at lakes with a range of fish densities. Fish densities are the mean number of sunfish (bluegill, pumpkinseed, hybrid and green sunfish) per trapnet set based on MN DNR fisheries surveys (2000-2002; Date provided). Below these results are results of historical fish surveys that correspond to weevil surveys from the same year in our regularly sampled lakes (summer-long average of bi-weekly weevil surveys, except Cenaiko when only one weevil survey was conducted in September 1998, one week prior to the fish survey).

Lake/Date	Date	Fish Density	Eggs	Larvae	Pupae	Adults	Total
Calhoun	7/24/00	241	0	0	0	0	0
Cedar	7/17/00	101	0	0.005	0	0	0.005
Bald Eagle	7/8/02	64	0	0	0	0.008	0.008
Peltier	8/5/02	60	0.042	0	0	0	0.042
Schultz	8/1/02	55	0	0	0	0.013	0.013
Centerville	7/29/02	35	0.218	0.066	0.019	0.042	0.346
Independence	7/23/01	28	0	0	0	0.014	0.014
Vadnais	7/16/01	20	0.169	0.013	0.025	0.113	0.319
Historical surveys							
Auburn	6/19/00	113	0.023	0.015	0.007	0.016	0.061
Cenaiko	9/9/98	5	0.856	1.978	0.156	0.611	3.600
Cenaiko	9/4/02	25	0.002	0.004	0.000	0.002	0.008
Otter	7/30/01	2	0.205	0.088	0.015	0.137	0.444
Otter	6/10/02	6	0.135	0.079	0.011	0.091	0.320

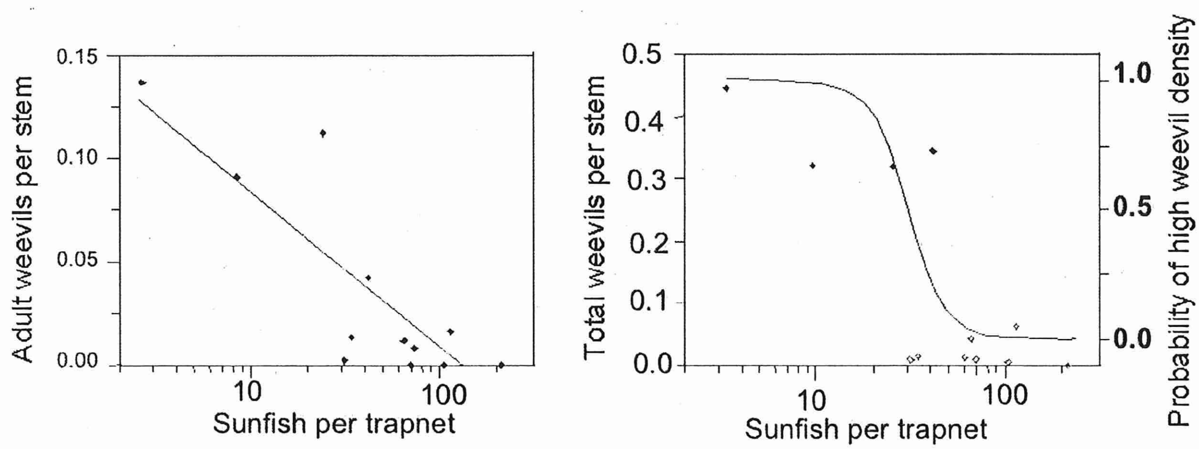

Fig. 7. Regression of adult weevil density on sunfish trapnet catch and logistic regression with total weevil density. Cenaiko Lake 1998 weevil densities were very high (Table 14) and were outliers and were dropped from the analysis.

Weevil Introduction/Manipulation:

To determine if we could stock weevils to enhance populations and get control of Eurasian watermilfoil, we stocked weevils into two Minneapolis lakes: Harriet (high sunfish density) and Hiawatha (low sunfish density). No weevils were found in stem surveys prior to stocking and no weevils were found in biomass samples taken immediately prior to stocking at either lake (Table 15). Weevils were found at both lakes after stocking (Table 15 and 17).

At Harriet, there was a significant increase in weevil abundance (per m^2 and per stem) after stocking in 2002 (Table 15; $p < 0.004$) but no difference between stocked and not-stocked plots. Stocking enhanced abundance, but weevils quickly moved beyond the stocked plots. Weevil densities increased through early September to 0.1 per stem in Harriet (Table 17). However, even though the plots were > 100m apart, weevils moved and colonized the not-stocked plots. Although a few weevil juveniles have been found in previous years in Lake Harriet, all adults since 2000 have been *Phytobius*, suggesting that milfoil weevil populations were very low in Lake Harriet prior to stocking in 2002. *Acentria* and *Parapoynx* were not found at Harriet.

In 2003, weevils were found in May and June prior to additional stocking, but the population did not increase even with stocking (Table 17). Only one weevil was found in the biomass samples, a pupa in the June 2003 not-stocked plot. Thus although Harriet attained a higher density of weevils after stocking in summer 2002 than Hiawatha, the population failed to increase in 2003, even with additional stocking. Stocking did appear to establish a low density of weevils at Harriet (Table 17) although it is not clear if the population will persist.

At Hiawatha, *Acentria* was present at low densities prior to stocking in 2002 but no milfoil weevils were found (Table 15). Weevils appeared after stocking in 2002 but densities were lower than Harriet and it was mid-September before weevils were common (Table 17). There was a significant increase in weevil abundance (per m^2 and per stem) after stocking (Table 15; $p < 0.1$), but no difference in weevil abundance between stocked and not stocked plots ($p>0.8$). These results suggest substantial within-lake movement of weevils within a summer and indicate that control and treatment plots should be placed very far apart (opposite sides of the lake).

In 2003, weevils were found at low densities in both the biomass and biweekly surveys; densities were similar between stocked and not-stocked plots (Tables 15 and 17). Densities were typically <0.2 per stem. There was no evidence of an additional increase in weevil density due to stocking in 2003 and it is likely a low-density population was established in both stocked and not-stocked plots. *Acentria* was much more abundant in 2003, particularly in the stocked plots (20-40/m^2). *Acentria* was rare in the biweekly surveys and its high occurrence in the biomass samples was likely because it was on non-milfoil plants. The overall higher density of weevils and caterpillars in Hiawatha compared to Harriet is consistent with lower sunfish predation and the lower density of sunfish found in Hiawatha (11/trapnet vs 340/trapnet at Harriet, MN DNR Lake Surveys). More study is required to determine if herbivore densities will persist or increase at Hiawatha.

Milfoil and total plant biomass was lower in Hiawatha than Harriet (perhaps due to clarity) and milfoil was more dominant in Harriet (Table 16). Significant declines of milfoil were not noted in either lake, but in 2002, milfoil increased significantly more in the not-stocked plots compared to stocked plots at Harriet (ANOVA of differences; $p <0.04$) while no significant change in non-milfoil biomass was detected ($p>0.8$). Overall, milfoil increased over the summer at Harriet and there was a significant ($p <0.07$) stocking by session interaction with the increase in milfoil at the not-stocked plots. The potential differences in milfoil among stocked and not-stocked plots did not carry over into 2003. Repeated measures ANOVA with the post stocking data found no significant difference in milfoil or non-milfoil biomass and not significant session by treatment interactions (all $p>0.5$). No significant differences in weevil densities were found either.

At Hiawatha, there was no effect of treatment on milfoil biomass and no change in milfoil biomass with treatment or date (all $p > 0.1$) in 2002 although milfoil biomass decreased in stocked plots and increased in unstocked plots. There was a significant decrease in non-watermilfoil biomass from June to September 2002 ($p<0.001$) and a significant decrease in number of species, both likely due to decreases in water clarity. A repeated measures ANOVA with the post stocking samples (September 2002, June and August 2003) indicated a significant site (treatment) effect ($p < 0.05$) on milfoil biomass, however, milfoil biomass was higher in the stocked plots. No significant time or time by plot interaction was found and no significant effects were found for total biomass. Native plants did increase over the study ($p < 0.1$) and the percentage of milfoil was lower in not-stocked plots. Thus no significant reduction in milfoil biomass was evident, however, weevils were distributed across stocked and not-stocked plots and may have prevented an increase in milfoil at Hiawatha (compare to Harriet) and may have contributed to the significant decrease in the control plots. Weevil densities in biweekly surveys were 50% higher in the not-stocked plots (0.15/stem) than the stocked plots (0.09/stem; Table 17), although the biomass samples showed less of a difference and a higher density per area in the stocked plots. An ANCOVA with weevil density (number per sample) as the covariate showed that weevil density was a significant covariate ($p<0.01$), although it is unclear how weevils were affecting the noted treatment effect.

It was somewhat surprising that adult weevil densities were similar in both lakes after stocking in 2002 and total weevil densities were higher in Harriet than in Hiawatha because Harriet has a much high density of sunfish (over 320/trapnet set in 2000) than Hiawatha (11/trapnet set in 2001). However, poor water quality and clarity in Hiawatha may have limited weevil success there during 2002. In 2003, weevil densities were similar in both lakes before restocking but adults became more common in Hiawatha as the summer progressed (Tables 15 and 17). The 2003 summer mean total weevil density was 0.12 per stem. The very low density in Harriet after early July 2003 and the absence of herbivores from the biomass samples in August suggests that herbivores will likely not persist in Harriet as long as the high sunfish density remains.

In summary, stocking did result in establishment of detectible weevils populations in both lakes that carried over to the next summer. Weevils may remain established at Hiawatha but it is less clear if they will persist at Harriet. The summer average weevil density in 2003 was 3 times higher in Hiawatha (0.12/stem) than Harriet (0.04/stem). Weevils dispersed into not-stocked areas and densities were not adequate to control the plants, although the fair population at Hiawatha in 2003 may have prevented the milfoil from increasing to higher density. Overall, however, there were no significant reductions of milfoil associated with weevil stocking in either lake. More time may be required to develop an adequate density of herbivores at Hiawatha. Predation by sunfish likely limited weevils at Harriet and future surveys should be conducted to determine if populations will persist in either lake.

Table 15. Abundance of weevil stages (N/m^2 and number per milfoil stem ± 2SE) and *Acentria* and *Parapoynx* before stocking (June and July) and after stocking (August and September) from biomass samples from stocked and not-stocked plots at Lakes Harriet and Hiawatha in 2002 and 2003. N = 12 samples from each plot and date.

Harriet Date	Weevil	Larvae N/m^2	Pupae N/m^2	Adults N/m^2	Total *E.l.* N/m^2	*Acentria* N/m^2	*Parapoynx* N/m^2
7/11/02	Stocked	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	per stem	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
	Not Stocked	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	per stem	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
9/14/02	Stocked	5.8±8.3	1.7±2.2	4.2±4.6	11.7±13.2	0.0±0.0	0.0±0.0
	per stem	0.014±0.016	0.006±0.009	0.018±0.023	0.038±0.031		
	Not Stocked	5.0±6.7	2.5±3.6	5.8±5.8	13.3±9.0	0.0±0.0	0.0±0.0
	per stem	0.012±0.016	0.013±0.022	0.023±0.022	0.047±0.037		
6/16/03	Stocked	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	per stem	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
	Not Stocked	0.0±0.0	0.8±1.7	0.0±0.0	0.8±1.7	0.0±0.0	0.0±0.0
	per stem	0.000±0.000	0.006±0.012	0.000±0.000	0.006±0.012		
8/25/03	Stocked	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	per stem	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
	Not Stocked	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	per stem	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		

Hiawatha Date	Weevil	Larvae N/m^2	Pupae N/m^2	Adults N/m^2	Total *E.l.* N/m^2	*Acentria* N/m^2	*Parapoynx* N/m^2
7/18/02	Stocked	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3.3±2.8	0.0±0.0
	per stem	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
	Not Stocked	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.7±2.8	0.0±0.0
	per stem	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
9/12/02	Stocked	0.0±0.0	0.0±0.0	5.0±8.0	5.0±8.0	2.0±4.0	0.0±0.0
	per stem	0.000±0.000	0.000±0.000	0.050±0.083	0.050±0.083		
	Not Stocked	1.0±2.0	0.0±0.0	3.0±4.3	4.0±6.1	0.0±0.0	0.0±0.0
	per stem	0.009±0.019	0.000±0.000	0.056±0.079	0.065±0.087		
6/27/03	Stocked	0.0±0.0	0.0±0.0	1.7±2.2	1.7±2.2	20.0±16.7	0.0±0.0
	per stem	0.000±0.000	0.000±0.000	0.019±0.028	0.019±0.028		
	Not Stocked	0.0±0.0	0.0±0.0	1.7±2.2	1.7±2.2	2.5±3.6	0.0±0.0
	per stem	0.000±0.000	0.000±.000	0.080±0.141	0.080±0.141		
8/28/03	Stocked	0.0±0.0	0.0±0.0	2.5±3.6	2.5±3.6	39.2±19.5	1.7±3.3
	per stem	0.000±0.000	0.000±0.000	0.034±0.049	0.034±0.049		
	Not Stocked	0.0±0.0	0.0±0.0	1.8±2.4	1.8±2.4	2.7±3.9	0.0±0.0
	per stem	0.000±0.000	0.000±0.000	0.047±0.066	0.047±0.066		

Table 17. Results of weevil surveys in stocked lakes Hiawatha and Harriet. Numbers are densities of weevil life stages (per stem), total weevils per stem and density (per stem) of the caterpillars *Acentria* (Acent) and *Parapoynx* (Parap). In 2003 additional weevils were stocked in mid-July.

Date	Treatment	Eggs	Larvae	Pupae	Adults	Total	*Acent*	*Parap*
Hiawatha								
7/30/02	stocked	0.000	0.000	0.000	0.013	0.013	0.009	0.000
7/30/02	notstocked	0.013	0.000	0.000	0.000	0.013	0.000	0.000
8/12/02	stocked	0.000	0.000	0.000	0.000	0.000	0.008	0.000
8/12/02	notstocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8/26/02	stocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8/26/02	notstocked	0.023	0.034	0.000	0.000	0.057	0.000	0.000
9/12/02	stocked	0.000	0.000	0.000	0.073	0.073	0.000	0.000
9/12/02	notstocked	0.000	0.000	0.000	0.072	0.072	0.000	0.000
2002	mean	0.005	0.004	0.000	0.020	0.029	0.002	0.000
5/23/03	stocked	0.021	0.000	0.000	0.000	0.021	0.000	0.000
5/23/03	notstocked	0.278	0.000	0.000	0.000	0.278	0.000	0.000
6/4/03	stocked	0.025	0.000	0.000	0.000	0.025	0.000	0.000
6/4/03	notstocked	0.029	0.057	0.000	0.000	0.086	0.000	0.000
6/17/03	stocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6/17/03	notstocked	0.000	0.089	0.000	0.056	0.144	0.000	0.000
7/2/03	stocked	0.056	0.000	0.000	0.000	0.056	0.000	0.000
7/2/03	notstocked	0.098	0.000	0.000	0.000	0.098	0.000	0.000
7/14/03	stocked	0.045	0.023	0.000	0.011	0.080	0.000	0.000
7/14/03	notstocked	0.167	0.000	0.000	0.028	0.194	0.000	0.000
7/31/03	stocked	0.162	0.083	0.000	0.021	0.266	0.021	0.000
7/31/03	notstocked	0.014	0.022	0.014	0.014	0.064	0.000	0.000
8/12/03	stocked	0.068	0.114	0.000	0.011	0.193	0.057	0.000
8/12/03	notstocked	0.064	0.076	0.000	0.030	0.170	0.021	0.000
2003	mean	0.073	0.033	0.001	0.012	0.120	0.007	0.000
Harriet								
7/24/02	stocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7/24/02	notstocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8/6/02	stocked	0.000	0.000	0.000	0.010	0.010	0.000	0.000
8/6/02	notstocked	0.104	0.000	0.000	0.000	0.104	0.000	0.000
8/19/02	stocked	0.031	0.000	0.021	0.014	0.066	0.000	0.000
8/19/02	notstocked	0.010	0.104	0.021	0.010	0.146	0.000	0.000
9/6/02	stocked	0.000	0.021	0.010	0.052	0.083	0.000	0.000
9/6/02	notstocked	0.063	0.000	0.000	0.045	0.107	0.000	0.000
9/17/02	stocked	0.000	0.021	0.000	0.021	0.042	0.000	0.000
9/17/02	notstocked	0.000	0.031	0.031	0.031	0.094	0.000	0.000
2002	mean	0.021	0.018	0.008	0.018	0.065	0.000	0.000
5/23/03	stocked	0.088	0.000	0.000	0.000	0.088	0.000	0.000
5/23/03	notstocked	0.227	0.000	0.000	0.023	0.250	0.000	0.000
6/4/03	stocked	0.057	0.000	0.000	0.000	0.057	0.000	0.000
6/4/03	notstocked	0.021	0.010	0.000	0.021	0.052	0.000	0.000
6/17/03	stocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6/17/03	notstocked	0.000	0.056	0.000	0.000	0.056	0.000	0.000
7/2/03	stocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7/2/03	notstocked	0.000	0.000	0.009	0.009	0.019	0.000	0.000
7/15/03	stocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7/15/03	notstocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7/30/03	stocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7/30/03	notstocked	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8/11/03	stocked	0.000	0.000	0.073	0.000	0.073	0.033	0.000
8/11/03	notstocked	0.000	0.010	0.000	0.010	0.021	0.000	0.000
2003	mean	0.028	0.005	0.006	0.005	0.044	0.002	0.000

Table 16. Total plant biomass (g dm/m^2, ±SE), milfoil biomass (MSP), non-milfoil biomass and percent milfoil before (July) and 7 weeks after stocking weevils in stocked and not-stocked plots at Hiawatha and Harriet.

Session	Date	Trt	Total Biomass	MSP	NonMSP	%MSP
Hiawatha	7/18/02	Stocked	77±23	38±21	39±18	42.7± 19.9%
	7/18/02	Not Stocked	99±40	18±16	81±40	19.0± 16.2%
	9/12/02	Stocked	39±24	29±24	10±11	52.6± 25.9%
	9/12/02	Not Stocked	37±15	22±14	15±8	55.0± 20.0%
	6/27/03	Stocked	135±103	103±93	32±28	66.5± 20.9%
	6/27/03	Not Stocked	86±85	51±86	33±22	22.6± 17.7%
	8/28/03	Stocked	92±47	55±24	36±29	66.8± 19.9%
	8/28/03	Not Stocked	62±35	18±17	43±28	28.8± 17.8%
Harriet	7/11/02	Stocked	336±133	319±143	16±19	84.2± 17.4%
	7/11/02	Not Stocked	170± 84	155± 85	14±10	88.0± 11.0%
	9/14/02	Stocked	339±123	308±114	31±26	92.3± 6.5%
	9/14/02	Not Stocked	371±128	367±126	4±3	98.7± 1.2%
	6/16/03	Stocked	275±138	264±135	11±15	95.4± 4.8%
	6/16/03	Not Stocked	289±133	272±121	18±35	87.8± 15.5%
	8/25/03	Stocked	271±114	253±110	18±22	89.8± 10.6%
	8/25/03	Not Stocked	130±251	211±126	39±40	79.1± 19.5%

Effects of plant community:

Plant manipulation plots were established in Otter Lake and Lake Auburn in 2001 and were resampled in 2002. A set of plots was established in Cedar Lake in 2002. Each manipulation consisted of twenty plots; five replicates each of 4 treatments (remove no plants (Control), remove all plants, remove milfoil, or remove native plants). Treatments were assigned to plots in a randomized block (by location) manner. In 2003, the removal plots in Cedar and Otter were resampled for biomass in late June or early July.

At Lake Auburn, the community was dominated by coontail (>90% of native biomass) and Eurasian watermilfoil (MSP) (Table 18). There were no significant differences in biomass or number of species prior to the manipulation (ANOVA, all $p > 0.2$).

Table 18. Mean biomass ± 2SE (g dry/m^2) of all plants (Total), Eurasian watermilfoil (MSP), all other plants (NAT) and the most common plants (coontail (CRT), flatstem pondweed (PZS), sago pondweed (PEC; now *Stuckenia pectinata*) and *Nymphaea* (NMP)) by treatment for the plant community manipulation at Lake Auburn 2001-2002. The percent of total plant biomass composed by MSP and percent of native plant mass composed of CRT along with the mean number of non-MSP species per sample (NSpec) are also given. Treatments were: No removal (Contr), Remove all plants (Remall), remove Eurasian watermilfoil (RemMSP) and remove all plants except MSP (RemNat). Plant manipulations occurred just after the initial sampling in June 2001. n = 5 plots per treatment.

Treat	Total	MSP	CRT	PZS	PEC	NMP	NAT	%MSP	%CRT	NSpec
6/13/01										
Contr	178.9	102.3	67.2	0.0	0.0	9.4	68.8	49.5%	95.9%	1.0
	55.3	75.7	50.4	0.0	0.0	18.8	57.7	25.8%	8.3%	0.3
Remall	239.4	118.0	101.0	0.1	0.0	20.3	121.4	45.6%	91.1%	1.3
	53.8	83.5	45.8	0.2	0.0	32.4	72.2	26.4%	9.6%	0.2
RemMSP	198.3	88.0	109.7	0.6	0.0	0.0	110.3	43.3%	99.8%	1.1
	38.8	38.2	67.0	1.3	0.0	0.0	68.1	23.5%	0.4%	0.2
RemNat	253.8	145.9	94.2	0.0	0.0	13.7	107.9	47.1%	86.2%	1.3
	84.2	94.9	65.4	0.0	0.0	12.4	60.4	23.5%	13.1%	0.2
9/21/01										
Contr	291.8	196.5	82.2	0.0	3.2	9.9	95.3	59.6%	77.6%	1.6
	126.6	150.3	63.8	0.0	4.2	13.1	55.3	24.6%	19.9%	0.4
Remall	104.8	5.7	91.0	0.3	0.0	7.8	99.1	11.3%	93.2%	1.3
	34.0	8.0	40.4	0.6	0.0	13.5	40.7	19.9%	8.2%	0.2
RemMSP	200.1	17.5	179.3	1.2	0.2	1.9	182.6	11.5%	97.7%	1.4
	74.6	15.8	72.3	2.4	0.4	3.8	71.3	10.6%	3.5%	0.6
RemNat	293.0	194.2	75.7	0.0	0.3	22.8	98.8	60.6%	72.4%	1.4
	106.8	157.1	91.0	0.0	0.4	27.5	83.7	34.0%	22.8%	0.5
6/13/02										
Contr	145.0	66.4	71.1	0.0	0.0	7.5	78.6	45.2%	96.1%	1.2
	53.9	62.4	64.8	0.0	0.0	15.0	77.5	38.0%	7.8%	0.4
Remall	154.6	64.9	88.1	0.2	0.0	0.0	89.8	51.4%	95.5%	1.3
	72.7	39.6	80.4	0.3	0.0	0.0	79.1	28.2%	9.0%	0.4
RemMSP	230.7	94.5	136.0	0.1	0.0	0.0	136.2	40.4%	98.3%	1.4
	124.7	76.9	106.0	0.3	0.0	0.0	105.8	14.9%	3.3%	0.2
RemNat	133.3	86.6	46.7	0.1	0.0	0.0	46.7	50.0%	99.4%	1.1
	77.6	58.1	27.2	0.1	0.0	0.0	27.2	23.1%	1.2%	0.4
9/20/02										
Contr	428.8	348.4	80.4	0.0	0.0	0.0	80.4	70.6%	100.0%	0.9
	176.6	189.1	83.0	0.0	0.0	0.0	83.0	33.2%	0.0%	0.2
Remall	231.8	82.6	137.7	0.0	0.0	11.4	149.2	42.9%	78.8%	1.3
	90.5	73.5	103.1	0.0	0.0	14.5	98.5	35.6%	23.2%	0.2
RemMSP	219.1	123.0	96.1	0.0	0.0	0.0	96.1	46.7%	100.0%	0.9
	123.5	129.5	61.0	0.0	0.0	0.0	61.0	35.5%	0.0%	0.2
RemNat	167.6	101.6	64.4	0.0	0.0	1.6	66.0	49.2%	97.5%	1.1
	124.2	111.2	46.4	0.0	0.0	3.2	46.3	23.8%	5.0%	0.5

Visual estimates of plant coverage confirm that the manipulations altered the community (Table 19; in July 2001 %MSP was lower in the Remove All and Remove-MSP treatments (Tukey's HSD, $p<0.01$) and % Natives was lower in Remove-All and Remove-Natives compared to the Remove-MSP treatment (Tukey's HSD, $p<0.05$). Repeated measures ANOVA with all sample dates indicated significant treatment effects for %MSP, %CRT, and %Native species (all $p < 0.01$), but not for other individual species or the mean number of species per plot. Significant session effects were found for MSP, %Natives and mean number of species (all $p<0.05$), but a significant session by treatment interaction was found only for %MSP ($p<0.05$). Milfoil increased, but remained reduced in the Remove-All and Remove-MSP treatments compared to the Remove-Natives treatment through 2001 (sessions 2 and 3), and continued to increase but did not differ by treatment in 2002 (sessions 4 and 5). Conversely, %CRT and %Natives were higher in the Remove-MSP treatment than the other treatments in session 2 and were higher in Remove-MSP than the Control and Remove-Natives in session 3 (Tukey's HSD, all $p<0.1$). In sessions 3 and 4, abundance of Natives remained higher in the Remove-All plots compared to Remove-Native plots (Tukey's HSD, all $p<0.1$). Native plants, predominantly CRT, quickly colonized the Remove-All plots and reduced the recovery of MSP until the fall of 2002. Removal of MSP allowed expansion of the natives in 2001, but by September of the second year milfoil recovered and was not dominated by the natives. Removal of natives favored Eurasian watermilfoil over natives, which remained suppressed through September 2002. As noted above, no changes in number of species were associated with the treatments.

The plant removals were also successful at manipulating the plant community biomass during the first summer; total plant biomass was reduced in the Remove-All treatment and milfoil biomass was reduced in the Remove-MSP treatment (Table 18). Overall, treatments resulted in significant changes in total dry biomass, MSP biomass, the percentage of MSP and coontail, and mean number of species (ANOVA, all $p<0.1$), but no significant changes in non-MSP biomass, coontail biomass or the mean number of non-watermilfoil species were detected in 2001. Coontail biomass increased (but not significantly) with removal of MSP and MSP increased substantially in both the Control and Remove-native treatments. In September, total biomass was lower in Remove-All than in the Control and Remove-Native treatments (Tukey's HSD, $p< 0.05$; the same was seen for MSP except $p=0.1$) and the percentage of MSP was lower in the Remove-All and Remove-MSP treatments than the Control and Remove-Native treatments (Tukey's HSD, $p< 0.05$). These results, consistent with visual estimates, suggest that coontail was able to quickly colonize and take advantage of removal of MSP and that proportional representation of MSP was reduced through the summer in the plots from which it was removed, however, MSP continued to dominate in the Control plots and the Remove-Natives plots. In the lower diversity and poorer water clarity system of Lake Auburn, Eurasian watermilfoil retained dominance in the Control or when natives were removed, but coontail was able to become dominant where Eurasian watermilfoil was removed, even in the Remove-All treatment.

Table 19. Visual estimates (2SE) of plant coverage of Eurasian watermilfoil (%MSP), all other plants (%NAT), the most common plants (coontail (%CRT), flatstem pondweed (%PZS), sago pondweed (%PEC; now *Stuckenia pectinata*) and *Nymphaea* (%NMP)) and the mean number of species by treatment for the plant community manipulation at Lake Auburn 2001-2002. Treatments were: No removal (Contr), remove all plants (RemAll), remove Eurasian watermilfoil (RemMSP) and remove all plants except MSP (RemNat). Plant manipulations occurred three weeks prior to the first visual estimate in June 2001. n = 5 plots per treatment.

Date	Treat	% MSP	% CRT	% PZS	% PEC	% NMP	%Nat	NSpp
7/9/01	Contr	43.9	37.0	0.1	2.5	4.0	43.6	3.0
		15.4	21.3	0.3	4.5	7.1	22.0	0.6
7/9/01	RemAll	5.6	25.6	0.6	0.0	0.9	27.1	2.8
		3.4	20.0	0.6	0.0	1.6	19.8	0.7
7/9/01	RemMSP	13.0	60.0	0.5	0.7	0.6	61.9	3.4
		7.9	14.9	0.5	1.4	0.8	15.6	1.0
7/9/01	RemNat	43.8	21.1	0.0	0.1	1.9	23.1	3.0
		12.4	6.3	0.0	0.2	2.1	6.3	0.6
8/2/01	Contr	43.7	40.0	0.1	2.6	3.7	46.4	3.6
		17.8	19.4	0.1	3.3	5.9	19.6	0.8
8/2/01	RemAll	18.5	43.4	0.4	0.4	2.0	46.3	3.2
		11.9	11.4	0.6	0.6	2.6	11.3	0.4
8/2/01	RemMSP	17.1	71.1	0.5	1.4	0.8	73.8	3.2
		10.8	13.2	0.6	2.2	1.3	14.5	1.2
8/2/01	RemNat	49.0	31.8	0.7	0.3	5.5	38.3	3.6
		16.0	13.5	1.0	0.3	5.5	13.8	1.0
9/21/01	Contr	44.0	34.3	0.0	4.5	10.4	49.1	3.0
		11.1	5.3	0.0	7.0	11.5	9.6	0.0
9/21/01	RemAll	20.1	54.8	0.3	0.8	6.6	62.4	2.8
		16.8	16.1	0.5	1.5	8.6	12.7	0.4
9/21/01	RemMSP	20.0	65.5	0.8	1.6	7.5	75.4	3.2
		6.6	18.6	1.5	2.4	11.2	15.5	1.2
9/21/01	RemNat	63.4	31.1	0.0	0.0	4.6	35.8	2.6
		14.4	15.6	0.0	0.0	3.9	14.4	0.5
7/22/02	Contr	11.6	24.6	0.0	0.0	0.4	25.0	2.2
		5.6	16.8	0.0	0.0	0.8	17.1	0.4
7/22/02	RemAll	17.3	56.2	0.0	0.0	3.0	59.2	2.6
		10.3	22.1	0.0	0.0	5.4	22.0	0.5
7/22/02	RemMSP	16.1	44.2	0.0	0.0	1.1	45.4	2.4
		6.4	24.7	0.0	0.0	1.7	25.0	0.5
7/22/02	RemNat	14.9	15.2	0.0	0.0	5.4	20.6	2.4
		9.1	10.7	0.0	0.0	6.7	12.0	0.5
9/4/02	Contr	38.5	36.6	0.3	0.0	0.5	37.4	2.6
		22.8	20.1	0.5	0.0	0.6	20.7	0.8
9/4/02	RemAll	20.6	49.8	0.0	0.0	1.9	51.6	2.2
		5.9	28.3	0.0	0.0	3.8	28.5	0.4
9/4/02	RemMSP	33.5	43.5	0.1	0.0	2.5	46.1	2.6
		17.4	17.4	0.3	0.0	3.3	18.1	0.8
9/4/02	RemNat	42.4	16.5	0.5	0.0	2.0	19.0	2.8
		31.9	22.0	1.0	0.0	2.6	25.3	0.7

In June 2002 biomass was lower at all plots than in June 2001, probably due to weather. However, MSP had recovered in the Remove-All and Remove-MSP plots (Table 18). To examine the longer-term effects of the manipulation, repeated measures ANOVA (treatments with repeated samples over time) was used to analyze the post manipulation (Sep 2001, June 2002, Sep 2002) data. Univariate results are only reported if the overall response was significant in the repeated measures analysis. Total biomass and MSP biomass both varied significantly by treatment ($p<0.01$), date ($p<0.1$) and the treatment by date interaction ($p<0.1$), however, no significant effects were found for coontail, non-MSP biomass, percentage milfoil or number of species. No significant treatment effects were found for any response variable in June 2002 but in September, MSP remained low in the Remove-All plots (Tukey's HSD, $p <0.05$). Although the mean number of non-MSP species declined throughout the experiment ($p<0.05$) there was no treatment effect or treatment by time interaction for number of species. Eurasian watermilfoil maintained its dominance in the Control and recovered in the Remove-MSP plots. Surprisingly, it did not increase its dominance in the Remove-Native and Remove-All plots; milfoil biomass was significantly lower than the Control at these plots in September 2002 (Tukey's HSD, both $p < 0.1$).

In September 2002 total biomass and MSP biomass were significantly related to pore water NH_4 (lower due to use), but there were no significant differences in exchangeable N with treatment and neither pore water or exchangeable N were significant covariates.

In this low clarity system, dominated by Eurasian watermilfoil and coontail, milfoil recovered from removal within a year and plants other than coontail failed to increase where Eurasian watermilfoil was reduced. This was not entirely due to a total lack of propagules, as *Stuckenia pectinata*, *Potamogeton zosteriformis* and *Nymphaea* were found at low levels in many plots, but clearly, environmental conditions, Eurasian watermilfoil and coontail prevented them from establishing significant populations after removal of some or all plants.

Otter Lake had a much more diverse plant community (Table 20) with 3 to 6 species (2-4 nonMSP species) per sample commonly collected. Coontail, although common, was typically < 15% of total plant biomass. Analysis of the pre-manipulation biomass indicated no differences associated with treatment plots (all $p> 0.1$). Date was a more significant factor in Otter Lake; total plant biomass declined significantly from June to September 2001 ($p < 0.001$) and this was primarily due to a significant decline in Eurasian watermilfoil from over 36 g/m^2 to less than 1 g/m^2 in September 2001. Non-Eurasian watermilfoil biomass also decreased significantly after our removal treatments, however, no significant differences in plant biomass due to treatment were found in 2001 with the exception of a significant increase in *Potamogeton richardsonii* in the Remove-MSP plots (Tukey's HSD, $p < 0.05$). The decline in milfoil was likely due to herbivore damage. In June 2001, weevil densities averaged $0.5/m^2$ and *Acentria* and *Parapoynx* averaged 1.5 and $1.25/m^2$ respectively, but by September *Acentria* and *Parapoynx* increased to 2.75 and $33/m^2$, It should be noted that the removal plots were distant from our regular transect sites and illustrate the lake-wide decline of Eurasian watermilfoil associated with herbivore damage. The percent contribution of Eurasian watermilfoil decreased and the percent coontail increased from June to September and the mean number of species also decreased over time (all $p < 0.05$), but no significant treatment effects were found for these variables. No significant differences among treatments in sediment nitrogen (pore water or exchangeable N), bulk density or percent organic matter were found for the September 2001 sediment cores.

Table 20. Mean biomass ± 2SE (g dry/m^2) of all plants (Total), Eurasian watermilfoil (MSP), all other plants (NAT) and the most common plants (coontail (CRT), *Elodea* (ELD), *Najas* (NAJ), flatstem pondweed (PZS), sago pondweed (PEC), *Potamogeton richardsonii* and *praelongus* (PRI) and *Chara* (CHA)) by treatment for the plant community manipulation at Otter Lake 2001-2003. The percent of total plant biomass composed by MSP and CRT along with the mean number of non-MSP species per sample (Spec) are also given. Treatments were: No removal (Contr), Remove all plants (Remall), remove Eurasian watermilfoil (RemMSP) and remove all plants except MSP (RemNat). Plant manipulations occurred just after the initial sampling in June 2001. n = 5 plots per treatment.

Treat	Total	MSP	CRT	ELD	PZS	NAJ	PEC	PRI	CHA	NAT	%Spic	%CRT	Spec
6/7/01													
Contr	144.2	43.2	24.5	34.2	14.3	2.2	0.0	5.3	20.5	97.5	36.9%	13.3%	4.8
	60.6	39.9	31.8	30.0	14.8	3.2	0.0	3.4	25.6	90.5	32.3%	10.3%	0.2
Remall	114.7	37.3	10.1	18.3	11.2	35.9	0.0	1.9	0.1	77.4	41.7%	8.1%	3.8
	74.1	22.5	8.4	25.7	11.8	55.2	0.0	2.4	0.1	71.8	29.1%	4.2%	1.1
RemMSP	114.2	36.4	18.8	32.7	21.7	3.1	0.0	0.0	1.5	77.8	40.8%	14.2%	3.8
	55.4	32.0	15.7	42.9	14.2	5.7	0.0	0.0	2.5	71.7	32.1%	7.8%	0.7
RemNat	192.7	130.2	13.6	19.6	15.4	1.1	0.0	0.0	12.7	62.5	68.2%	7.9%	4.1
	128.0	120.2	18.4	33.4	19.0	1.1	0.0	0.0	25.1	65.9	25.0%	8.2%	0.2
9/20/01													
Contr	60.4	0.3	12.0	16.2	2.2	13.1	0.7	1.1	11.7	60.1	0.4%	28.0%	3.7
	37.5	0.6	11.3	22.1	2.2	13.5	1.4	2.2	23.4	37.5	0.7%	17.2%	0.7
Remall	15.7	0.3	5.5	5.2	1.7	1.6	0.0	0.4	0.0	15.4	2.0%	25.5%	3.0
	11.9	0.6	6.4	6.4	2.2	2.0	0.0	0.8	0.0	12.0	4.1%	21.0%	0.7
RemMSP	53.6	0.1	14.1	15.0	4.0	13.1	3.5	3.0	0.1	53.5	0.1%	26.8%	3.5
	43.4	0.1	10.2	8.1	2.5	16.1	7.0	5.5	0.2	43.3	0.1%	14.5%	1.6
RemNat	41.3	0.2	2.6	9.9	2.5	14.2	4.3	1.2	3.8	41.1	0.5%	11.5%	3.6
	28.1	0.4	1.7	9.4	3.8	15.7	7.9	1.0	7.6	28.1	1.0%	13.0%	0.9
6/11/02													
Contr	73.9	12.4	3.2	56.3	0.0	0.4	0.0	0.3	1.3	61.5	16.5%	11.7%	1.9
	39.3	17.7	3.3	36.1	0.1	0.8	0.0	0.6	1.7	34.6	19.1%	19.3%	0.8
Remall	121.0	9.6	9.2	45.5	0.0	14.4	0.0	0.0	37.5	111.4	9.8%	5.4%	2.0
	50.9	18.7	18.4	38.0	0.0	28.8	0.0	0.0	20.1	58.5	18.4%	10.7%	0.8
RemMSP	70.1	0.4	17.8	29.6	1.9	18.6	0.0	0.0	1.5	69.7	0.7%	10.7%	2.2
	25.7	0.8	34.1	23.8	3.5	22.8	0.0	0.0	2.3	26.2	1.3%	19.3%	1.0
RemNat	88.7	2.4	2.7	61.5	0.7	9.5	0.0	0.5	9.2	86.3	3.6%	1.7%	2.4
	33.6	2.1	3.3	41.2	0.9	16.6	0.0	1.0	18.4	34.2	3.9%	1.7%	1.0
9/13/02													
Contr	97.9	0.1	4.2	64.4	5.7	4.9	8.1	4.1	6.1	97.8	0.2%	5.5%	4.1
	71.4	0.1	4.3	71.6	8.0	4.3	16.2	7.4	7.5	71.4	0.3%	7.0%	1.0
Remall	68.5	0.1	5.7	27.0	0.3	15.8	0.0	6.8	6.4	68.4	2.3%	12.2%	2.8
	57.3	0.1	7.0	35.3	0.3	31.1	0.0	8.9	11.4	57.3	4.5%	19.4%	0.7
RemMSP	113.9	0.1	8.9	75.4	0.2	24.9	0.2	0.0	0.0	113.8	0.0%	7.3%	3.6
	68.0	0.1	6.8	40.7	0.3	41.3	0.4	0.0	0.0	68.0	0.1%	5.5%	1.2
RemNat	145.1	0.5	0.6	105.3	1.0	18.4	0.0	0.6	14.7	144.6	1.0%	0.3%	4.2
	68.7	1.0	0.5	74.7	2.0	33.6	0.0	1.2	28.7	68.8	2.1%	0.3%	1.1

Table 20 Continued

Treat	Total	MSP	CRT	ELD	PZS	NAJ	PEC	PRI	CHA	NAT	%Spic	%CRT	Spec
6/18/03													
Contr	52.4	9.7	0.0	38.1	0.7	0.0	0.0	0.0	0.2	42.6	18.3%	0.4%	2.6
	44.2	14.2	0.1	49.6	0.8	0.0	0.1	0.0	0.3	48.4	25.0%	0.7%	0.4
Remall	74.1	0.2	0.2	58.5	0.4	0.0	0.0	0.0	13.0	73.0	0.3%	0.5%	2.5
	73.7	0.2	0.4	78.9	0.5	0.0	0.0	0.0	26.0	74.2	0.5%	0.7%	1.3
RemMSP	101.4	0.6	28.9	68.9	2.4	0.0	0.0	0.0	0.6	100.8	0.4%	15.1%	2.7
	77.6	1.0	51.2	31.0	1.2	0.0	0.0	0.0	1.2	76.7	0.5%	19.5%	0.6
RemNat	201.2	0.6	0.4	127.1	0.4	0.0	0.0	0.0	39.1	200.4	0.4%	0.3%	2.1
	103.5	1.0	0.5	110.2	0.4	0.0	0.1	0.0	78.2	103.8	0.6%	0.5%	0.4

Visual estimates of coverage three weeks after manipulations show that milfoil was reduced in the Remove-MSP and Remove-All plots (<2% coverage) and was highest Control and Remove-Native treatments (Table 21; Tukey's HSD, all $p < 0.07$). Native species coverage was highest in the Remove-MSP and Control plots and significantly reduced in the Remove-All treatment (Tukey's HSD, all $p < 0.01$). Repeated measures ANOVA indicated significant treatment effects for Eurasian watermilfoil and significant treatment by date interactions for Eurasian watermilfoil and total native plants (all $p < 0.05$), but not for other taxa or the mean number of species per plot. Most taxa showed significant changes over time. When the last session was dropped (due to loss of 3 replicates), repeated measures ANOVA indicated significant treatment effects for Eurasian watermilfoil, sago pondweed, broad-leafed *Potamogeton*, and total native plants ($p \leq 0.1$) and significant treatment by date interactions for Eurasian watermilfoil and native plants ($p<0.05$). Broad leafed *Potamogetons* (*P. amplifolius, richardsonii, robbinsii, gramineus* and *praelongus*) remained highest in Control plots (Tukey's HSD, all $p < 0.05$), but sago pondweed was more abundant in the Remove-Native plots than Remove-MSP plots (Tukey's HSD), suggesting that it had been suppressed by other native plants. Eurasian watermilfoil coverage was highest in the remove native plots and native plant coverage was lower in Remove-All compared to the Controls and Remove-MSP treatments (Tukey's HSD, all $p < 0.05$). Eurasian watermilfoil remained suppressed in the Remove-All and Remove-MSP plots over time and decreased after early July in the Control and Remove-Native plots (Fig. 8); the suppression was due to herbivore damage. Native plants remained relatively constant in the Control and Remove-MSP plots, but increased in the Remove-All and Remove-Native plots, recovering to premanipulation levels by late 2001 or early 2003. Because Eurasian watermilfoil was already at low density and suppressed by herbivores, no significant increase in native plants was noted in the Remove-MSP treatment relative to the Control. The recovery of native plants in the Remove-All and Remove-Native plots was not due to any single species. *Elodea* and coontail were initially dominant, followed by *Najas*, which became dominant in September 2001 (Table 21). While *Elodea* continued to increase in 2002, coontail and *Najas* decreased. The mean number of species and native species declined over time ($p< 0.001$) in all plots (from 7 in July 2001 to <4 in September 2002), but no significant treatment or treatment by time interactions were found.

As reflected in the visual surveys, Eurasian watermilfoil biomass remained suppressed in all treatments in 2002, again due to suppression by herbivores. Milfoil was apparently too rare to support detectible weevil populations, but low densities of *Acentria* (0.3 ± 0.5 /m^2) and *Parapoynx* (4.3 ± 2.9 /m^2) were found in June, probably associated with native plants. Perhaps because of the low Eurasian density, few significant treatment effects were noted for biomass after the manipulation. Other than a significant decline of Eurasian watermilfoil and percent milfoil between June and September 2002 and a significant increase in total and non-watermilfoil biomass during the same time, the only treatment effect was for *Chara*, due mainly to its abundance in Remove-All plots in June 2002. Repeated measures analyses of all post removal samples (Sep 2001, June 2002, Sep 2002 and June 2003) revealed few significant treatment effects. Coontail was affected by treatment ($p<0.05$); it was higher in Remove-MSP plots. Total biomass, non-MSP biomass and *Chara* showed date by time interactions. Most other measures showed no effects or a significant date effect (MSP, %MSP, *Elodea*, number of species). For example, *Elodea* increased and %CRT decreased throughout the study and the number of native species was higher in September than June samples. Conversely, Eurasian watermilfoil was more abundant in June than in September (perhaps due to summer suppression by herbivores). Native plant biomass had apparently reached an equilibrium prior to the removals and the suppression of Eurasian watermilfoil by milfoil weevils eliminated it as a competitive factor after June 2001.

Analysis of sediment in September 2001 and 2002 showed no overall effects of treatment on sediment (bulk density, %organic, exchangeable N and pore water ammonium), but pore water ammonium was significantly lower in 2002. Analysis of treatment effects on total and native plant biomass in September 2001 and 2002 with sediment nitrogen as a covariate resulted in some significant treatment effects that were not otherwise evident. Pore water ammonium and exchangeable nitrogen were significant covariates (they were significantly correlated, $r=0.64$) and inclusion of either as a covariate resulted in significant treatment effects with total and native plant biomass; with single species, these covariates were not significant and did not result in significant treatment effects for any single species, however. Total and native plant biomass increased with nitrogen and given the nutrient levels, Remove-MSP had higher native biomass and Remove-All had lower biomass given nitrogen levels.

Table 21. Visual estimates (2SE) of plant coverage of Eurasian watermilfoil (MSP), all other plants (NAT) and the most common plants (coontail (CRT), *Elodea* (ELD), *Najas* (NAJ), *Zosterella dubia* (HET), flatstem pondweed (PZS), sago pondweed (PEC; now *Stuckenia pectinata*), Broad leaf Potamogetons (*P. amplifolius, richardsonii, robbinsii, gramineus* and *praelongus* (BroadP)) and *Chara* (CHA)) by treatment for the plant community manipulation at Otter Lake 2001-2002. The mean number of species (NoSpp) and non-MSP species per plot (NatSp) are also given. Treatments were: No removal (Contr), Remove all plants (RemAll), remove Eurasian watermilfoil (RemMSP) and remove all plants except MSP (RemNat). n = 5 plots per treatment.

Treat	MSP	CRT	ELD	PZS	NAJ	HET	PEC	BroadP	CHA	NAT	Nospp	NatSp
7/6/01												
Contr	13.9	16.0	24.3	4.6	8.9	4.1	2.4	6.2	0.0	66.6	8.2	7.2
	9.3	7.0	15.9	2.1	9.5	2.6	2.3	3.1	0.0	17.6	1.7	1.7
RemAll	1.3	3.8	2.4	0.9	2.2	4.8	0.4	0.4	0.0	15.0	6.8	6.2
	2.0	3.3	1.8	0.8	3.3	1.3	0.8	0.2	0.0	5.3	1.2	0.7
RemMSP	1.6	20.4	22.4	7.0	15.8	7.7	0.3	3.3	0.3	77.9	8.0	7.0
	0.8	17.8	14.0	4.2	15.7	6.2	0.5	3.0	0.3	22.5	1.1	1.1
RemNat	23.5	8.7	7.8	4.3	5.6	7.8	0.3	1.4	4.4	41.1	8.4	7.4
	8.4	6.7	7.9	0.4	5.2	3.3	0.5	1.2	5.9	9.7	0.8	0.8
7/25/01												
Contr	2.8	12.1	24.5	9.0	7.6	2.4	1.0	5.9	2.5	65.4	8.6	7.6
	1.2	7.7	19.6	6.9	8.9	1.8	1.7	2.3	3.1	26.1	0.5	0.5
RemAll	1.1	12.3	6.3	4.1	7.4	4.1	1.1	0.8	4.6	41.5	7.6	6.8
	1.5	9.8	5.9	3.1	7.9	3.5	1.2	1.3	8.6	22.0	1.4	1.3
RemMSP	0.4	24.8	17.4	9.1	13.8	2.6	0.5	1.3	2.1	72.2	7.8	7.2
	0.5	23.5	11.3	7.8	15.8	3.8	0.6	1.0	2.5	31.6	1.7	1.3
RemNat	6.7	15.6	11.9	4.2	7.6	5.9	2.2	1.2	7.4	58.8	8.8	7.8
	3.6	13.0	12.9	2.6	5.6	6.3	2.8	1.0	11.6	20.9	2.0	2.0
8/14/01												
Contr	1.8	21.4	13.8	7.4	16.1	0.8	2.3	3.6	0.1	65.9	7.0	6.2
	1.8	14.7	10.8	7.3	18.2	0.8	2.5	2.6	0.3	22.9	2.1	2.0
RemAll	0.5	11.8	7.6	2.3	10.8	2.4	0.8	0.5	0.0	36.5	5.2	4.8
	0.7	10.2	6.6	2.6	13.6	2.9	0.7	0.7	0.0	30.1	2.8	2.6
RemMSP	1.6	21.8	18.4	4.6	18.6	4.2	0.8	0.9	0.0	70.1	7.6	7.0
	1.9	13.3	11.2	5.0	19.7	5.3	0.8	0.6	0.0	21.8	1.6	1.4
RemNat	4.1	19.6	10.3	3.1	17.8	2.2	3.2	0.6	0.0	56.9	6.6	5.6
	2.4	11.7	12.7	3.4	11.1	1.8	1.3	1.1	0.0	14.9	1.0	1.0
9/19/01												
Contr	2.6	18.4	20.8	7.5	25.9	1.8	1.3	4.8	0.0	80.8	7.8	7.0
	2.8	15.8	10.6	5.8	24.3	1.4	1.6	3.5	0.0	23.7	1.9	2.1
RemAll	3.4	5.9	8.9	1.4	34.4	5.9	1.1	2.4	0.0	60.5	6.2	5.4
	3.3	7.5	12.5	1.3	21.5	3.7	1.2	3.0	0.0	29.4	2.1	1.7
RemMSP	0.8	13.8	23.1	8.6	29.3	7.6	0.6	1.0	0.0	84.6	7.0	6.2
	0.6	10.0	14.4	4.9	12.2	13.1	1.3	0.6	0.0	20.3	0.6	0.7
RemNat	9.3	8.0	17.0	3.0	28.5	7.0	5.3	1.3	5.3	77.0	7.6	6.6
	7.0	4.8	14.4	2.3	21.1	2.8	5.6	1.6	10.5	15.8	1.0	1.0
7/23/02												
Contr	3.3	10.3	34.0	3.4	0.0	1.5	5.1	3.7	1.8	65.7	5.6	5.2
	5.9	10.9	17.7	5.3	0.0	3.0	4.4	4.8	3.5	16.6	1.0	1.2
RemAll	2.1	3.1	38.5	3.4	1.0	0.0	5.3	1.4	13.5	70.4	4.8	4.4
	3.9	3.2	35.2	4.1	2.0	0.0	8.2	2.1	17.0	24.0	0.7	1.2
RemMSP	0.8	7.4	45.8	4.0	1.9	0.3	2.0	5.0	4.6	73.8	5.6	5.2
	1.2	8.1	14.3	4.5	3.8	0.5	1.6	9.4	4.8	17.5	0.8	0.4
RemNat	4.0	5.0	51.1	2.8	6.3	2.0	7.9	0.4	0.8	78.0	5.2	4.8
	6.8	8.3	21.1	4.3	12.5	4.0	5.4	0.5	1.0	21.5	1.2	1.2

Table 21. continued

Treat	MSP	CRT	ELD	PZS	NAJ	HET	PEC	BroadP	CHA	NAT	Nospp	NatSp
9/9/02												
Contr	3.3	6.9	35.4	4.4	0.0	0.0	0.2	3.0	16.4	71.5	4.8	4.0
	2.5	7.4	25.8	3.4	0.0	0.0	0.4	4.8	16.8	18.4	0.7	0.9
RemAll	1.0	5.1	24.1	1.3	0.0	0.0	0.0	1.3	26.8	74.8	4.0	3.4
	0.9	3.5	34.6	1.1	0.0	0.0	0.0	1.9	32.3	34.0	0.9	1.0
RemMSP	0.3	6.1	49.6	4.4	0.0	0.1	0.0	1.0	14.8	77.1	4.4	4.2
	0.5	4.8	18.0	4.4	0.0	0.2	0.0	1.7	28.3	22.6	1.9	1.6
RemNat	0.0	2.5	63.8	4.4	0.0	0.0	0.3	1.3	0.6	74.0	1.8	1.8
	0.0	0.0	47.5	8.8	0.0	0.0	0.5	2.5	1.3	52.0	2.2	2.2

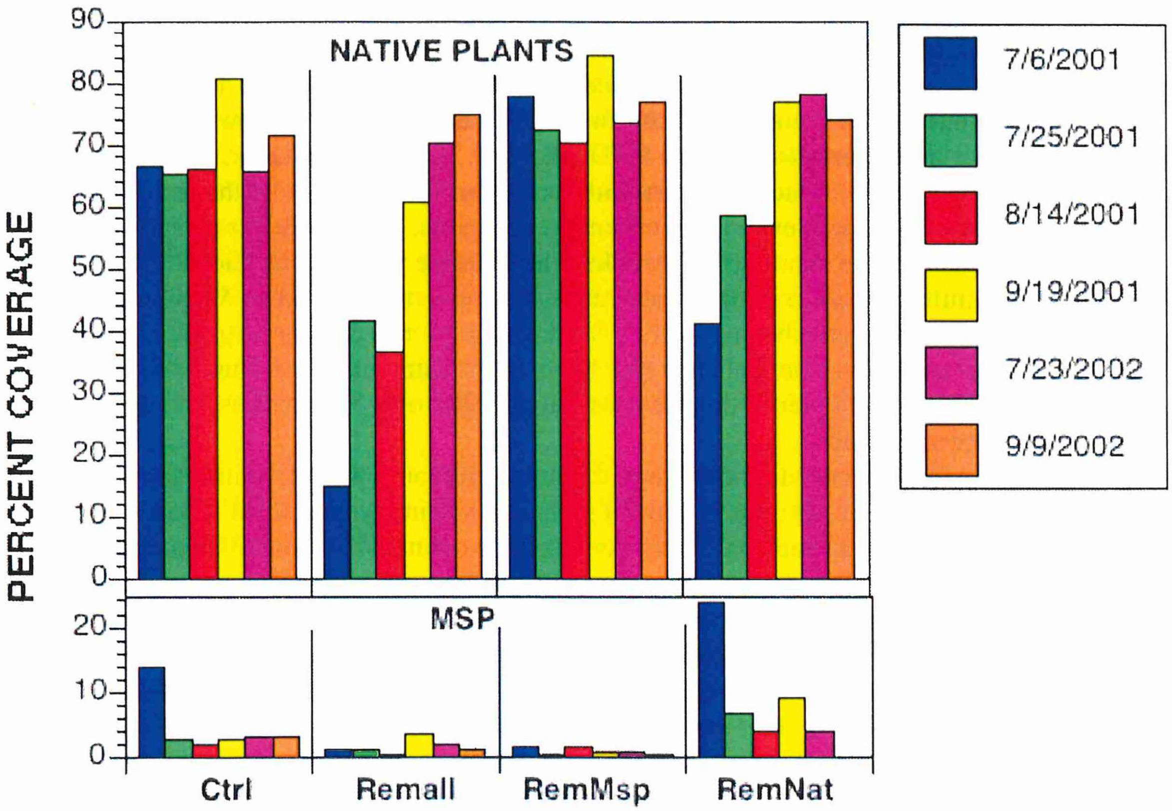

Fig. 8. Visual estimates of coverage of native plants and Eurasian watermilfoil (MSP) in the Otter Lake removal plots.

At Cedar Lake, removal manipulations were initiated in June 2002. Eurasian watermilfoil and coontail were the dominant taxa followed by some *Nymphaea* (Table 22). No differences in response variables were found among treatment plots prior to removal. Removals were successful and reducing total biomass and number of milfoil stems (both $p<0.1$) and milfoil biomass ($p<0.05$) in remove-all and remove-MSP plots (ANOVA of differences) but no reductions in natives were seen in September in the Remove-Native plots. This is probably due to rapid colonization by the unrooted coontail and by new shoots of *Nymphaea* from tubers (plants were pulled with roots but tubers were not removed from the plots). No treatment effects were found for native plant biomass, percentage of milfoil or coontail or number of species. Visual estimates of coverage also showed a reduction of plant coverage with removals (Table 23). Repeated measures ANOVA indicated significant treatment effects on milfoil coverage ($p<0.01$) but no seasonal effect or treatment by date interaction. Interestingly, milfoil coverage was significant lower in Remove-All plots compared to the Control and Remove-Native plots (Tukey's HSD, all $p<0.05$). No significant differences in coontail or number of species due to treatment or session were found.

Repeated measures ANOVAs on biomass confirmed these results; significant treatment effects were found for total biomass and milfoil biomass ($p<0.05$), but no treatment effects were found for native plant biomass, percentage of milfoil or coontail or number of species. Repeated measures analysis with the post-removal data indicated the same response; there were significant treatment effects on milfoil and total biomass but not on the other variables. The number of species did significantly increase from fall 2002. Eurasian watermilfoil was reduced in the Remove-All treatments relative to the Control and Remove-Native treatments however this effect did not continue in 2003; analysis of the July 2003 data revealed no significant treatment effects for milfoil or total biomass (Tukey's HSD, all $p > 0.1$). In Cedar Lake, removal of all plants and milfoil resulted in reductions in milfoil during the first year but by the second year, milfoil had recovered, although less so in the Remove-All plots. Although Eurasian watermilfoil became more abundant in the Remove-Native plots the increase was not significant. It is unclear why coontail and milfoil failed to return to pre-removal levels in the Remove-All plots, however, shading by *Nymphaea* may have been a factor. No differences in sediment (organics, bulk density, pore water ammonium or exchangeable N) among treatments were found but pore water ammonium was about 50% higher in the Remove-All and Remove-Native plots, likely due to less uptake by the fewer plants.

Overall, the manipulations did not reveal dramatic shifts or competitive interactions. Coontail tended to move into the remove milfoil plots but within a year milfoil recovered (in Otter Lake, other native plants such as *Elodea* replaced the coontail). Coontail also rapidly colonized the Remove-All plots, but within a year milfoil again became dominant, with the exception of Otter Lake, where it was controlled by herbivores. Except in Otter Lake, rooted native plants did not show a strong response to milfoil removal. Somewhat surprisingly, milfoil did not respond rapidly in the Remove-All plots; apparently due to its need to develop an extensive root system, milfoil is slow to recover from removal however the lack of a response by rooted natives enabled it to again become dominant a year or more after removal. It is possible that the longer suppression in Remove-All plots compared to Remove-MSP plots was due to a more complete removal of all plants in Remove-All compared to the Remove-MSP plots where we tried not to disturb other plants and may have left more milfoil roots.

Table 22. Mean biomass ± 2SE (g dryt/m^2) of all plants (Total), Eurasian watermilfoil (MSP), all other plants (NAT) and the most common plants (coontail (CRT), and *Nymphaea* (NMP)) by treatment for the plant community manipulation at Cedar Lake 2002-2003. The percent of total plant biomass composed by MSP and CRT along with the mean number of species (Spec) and non-MSP species per sample (NSpec) are also given. Treatments were: No removal (Contr), Remove all plants (Remall), Remove Eurasian watermilfoil (RemMSP) and Remove all plants except MSP (RemNat). Plant manipulations occurred just after the initial sampling in June 2002. n = 5 plots per treatment.

Treat	Total	MSP	CRT	NMP	NAT	%MSP	%CRT	Stems	Spec	NSpec
6/10/02										
Contr	187.1	109.9	70.0	5.3	77.2	58.2%	35.4%	182.0	2.3	1.3
	106.9	78.5	70.4	10.6	67.5	32.7%	25.9%	97.5	0.7	0.7
Remall	201.6	181.5	14.5	5.5	20.2	80.9%	16.2%	207.0	2.1	1.0
	120.0	121.9	17.2	11.0	22.2	17.7%	17.0%	111.4	0.6	0.7
RemMSP	167.9	124.8	37.3	5.8	43.1	78.7%	19.3%	204.0	1.7	0.7
	37.7	55.6	59.3	11.6	56.8	31.7%	32.7%	112.0	0.4	0.4
RemNat	139.0	127.7	11.3	0.0	11.3	93.4%	6.6%	171.0	1.4	0.4
	62.1	50.8	19.7	0.0	19.7	8.5%	8.5%	60.8	0.5	0.5
9/5/02										
Contr	319.9	222.4	97.5	0.0	97.5	76.0%	24.0%	189.0	1.8	0.8
	155.0	86.9	121.6	0.0	121.6	24.2%	24.2%	54.3	0.2	0.2
Remall	95.3	28.8	44.5	22.0	66.5	59.2%	31.4%	44.0	1.7	0.7
	103.1	40.4	65.6	44.0	68.3	33.7%	29.0%	36.2	0.6	0.6
RemMSP	87.7	45.7	38.3	3.7	42.0	73.5%	20.9%	84.0	1.5	0.5
	57.6	29.4	73.6	7.4	72.3	37.0%	37.6%	37.9	0.4	0.4
RemNat	219.2	170.5	30.3	18.4	48.7	82.4%	14.2%	137.0	1.4	0.4
	99.4	114.1	60.6	36.8	97.4	35.2%	28.4%	81.7	0.6	0.6
7/9/03										
Contr	266.2	223.9	35.1	5.4	42.0	64.3%	26.3%	156.3	2.0	1.0
	168.8	201.5	54.2	10.8	52.3	41.5%	37.9%	130.9	0.6	0.6
Remall	140.3	96.2	6.2	37.0	44.2	52.5%	19.7%	81.7	2.2	1.3
	166.5	185.8	8.0	74.0	67.7	40.5%	18.8%	133.5	0.9	0.7
RemMSP	278.0	205.4	54.8	16.8	72.6	68.7%	27.4%	78.0	2.0	1.0
	168.4	200.1	88.1	33.6	84.9	34.0%	36.1%	48.6	0.3	0.3
RemNat	309.4	277.6	31.7	0.0	31.8	81.9%	18.1%	152.0	1.8	0.8
	141.6	171.6	40.1	0.0	40.1	28.2%	28.3%	86.6	0.5	0.5

Table 23. Visual estimates (2SE) of plant coverage (%) of Eurasian watermilfoil (MSP), and the most common plants (coontail (CRT), *Potamogeton crispus* (PCR), sago pondweed (PEC; now *Stuckenia pectinata*),and *Nymphaea* (NMP)) by treatment for the plant community manipulation at Cedar Lake 2002. The mean number of species per plot (NoSp) is also given. Treatments were: No removal (Contr), Remove all plants (RemAll), remove Eurasian watermilfoil (RemMSP) and remove all plants except MSP (RemNat). n = 5 plots per treatment.

Date	Treatment	MSP	CRT	PCR	PEC	NMP	NoSp
6/28/02	Contr	47.3	5.6	0.4	0.0	5.5	2.2
		27.6	10.6	0.8	0.0	8.2	1.0
6/28/02	RemAll	16.3	2.9	0.5	0.0	0.3	1.6
		26.4	4.6	1.0	0.0	0.5	1.0
6/28/02	RemMSP	26.5	1.9	0.6	0.0	1.3	2.2
		22.6	1.7	1.3	0.0	2.5	0.4
6/28/02	RemNat	43.0	0.4	0.6	0.0	0.4	1.8
		28.0	0.8	1.3	0.0	0.7	1.2
7/29/02	Contr	39.9	11.9	0.0	1.0	4.5	2.0
		31.6	22.8	0.0	2.0	5.5	0.6
7/29/02	RemAll	6.7	1.3	0.0	0.0	0.6	1.8
		2.7	1.4	0.0	0.0	1.1	0.7
7/29/02	RemMSP	15.2	19.3	0.0	0.0	0.8	1.6
		19.7	36.0	0.0	0.0	1.6	1.0
7/29/02	RemNat	58.3	12.5	0.0	0.0	2.0	1.4
		34.5	25.0	0.0	0.0	4.0	0.8
8/9/02	Contr	58.8	12.9	0.0	0.0	6.9	2.2
		26.8	10.5	0.0	0.0	13.8	1.0
8/9/02	RemAll	4.6	1.6	0.0	0.0	1.3	2.0
		2.6	1.5	0.0	0.0	2.5	0.6
8/9/02	RemMSP	32.4	13.3	0.0	0.0	0.8	2.2
		20.4	15.2	0.0	0.0	1.5	0.4
8/9/02	RemNat	67.4	0.1	0.0	0.0	0.0	1.2
		30.9	0.3	0.0	0.0	0.0	0.4

Relationship of plant community to sediment characteristics:

McComas (1999) proposed that sediment nitrogen may be a good predictor of nuisance levels of Eurasian watermilfoil; high nitrogen sites should support dense growths of milfoil while lower nitrogen sites would be more amenable to native plants that are adapted to lower nitrogen levels. At low nitrogen sites, Eurasian watermilfoil should not reach nuisance levels. Recently, McComas (2003) updated his predictions and predicted that nuisance milfoil should occur in sediments with > 6ppm exchangeable ammonia. This prediction was based on a volume basis (mg/cm^3, McComas, personal communication). In 2001 we started measuring exchangeable (KCL extractable ammonium) N from the sediments because pore water ammonium is rapidly influenced by short-term plant uptake and may not reflect longer-term nitrogen availability. We analyzed all the sediment samples from 2001-2003 for exchangeable N and present analyses at three scales. Although our measures based on dry mass (mg N/g dm sediment) are not directly comparable to McComas's, they should provide some basis for testing his hypothesis and an assessment of possible N limitation of milfoil at our sites.

Mean total exchangeable N (mg N/g dry sediment) ranged from ≤ 0.005 (occasions at Cenaiko, Hiawatha and Vadnais) to > 0.1 mg/g (occasions at Otter and Cedar) (Table 24). Almost all individual sample values (95% of 378) were above the threshold of approximately 0.001 mg/g, which is not surprising as all sites have supported nuisance growths of Eurasian watermilfoil. Pore water ammonium typically contributed a small percentage of the total exchangeable N (compare KCL N in mg/kg to total exchangeable N in mg/g). As addressed below, pore water ammonium is more likely affected directly by plant density and uptake and exchangeable N might better reflect longer-term nutrient availability.

Among the lakes Cedar and Otter had high exchangeable N (ca. 0.08 mg/g), Auburn, Isles and Smiths had intermediate levels (ca. 0.05 mg/g) and Calhoun, Cenaiko, Harriet and Hiawatha had low exchangeable N (≤0.02 mg/g). This might explain the relatively low biomass at Hiawatha, however, lakes with low or intermediate levels of exchangeable N (e.g., Harriet, Auburn and Smith's Bay) often had equal or higher densities of milfoil than Cedar and Otter. Furthermore, the two lakes with clear milfoil declines, Otter and Cenaiko, represent opposite ends of sediment fertility, suggesting that herbivore induced declines are not limited to poor or highly fertile sites.

Table 24. Sediment bulk density (g/mL), % organic matter, pore water NH_4+ (mgN/L), KCL extracted N (ppm, less pore water)) and total exchangeable N (mg N/ g dry sediment) Values are means (2SEs) of typically 9 samples, three shallow, three intermediate and three deep at each site.

Lake	Date	Density	% Organic	NH_4	KCL ext N	Total Exch N
Auburn	6/15/01	0.50	11.23	0.98	72.85	0.0745
		0.18	4.23	0.38	20.81	0.0215
	7/17/01	0.57	25.69	3.72	38.67	0.0448
		0.26	30.49	1.92	17.55	0.0212
	8/29/01	0.47	10.90	5.46	42.99	0.0551
		0.18	3.77	1.11	15.47	0.0227
	6/27/02	0.53	18.83	6.61	47.34	0.0585
		0.12	6.27	3.25	25.97	0.0391
	9/6/02	0.62	19.70	5.14	32.77	0.0332
		0.22	10.41	.	12.67	0.0126
	8/29/03	0.35	11.29	3.71	48.78	0.0570
		0.10	3.49	1.86	16.56	0.0209
Calhoun	6/28/01	0.68	6.02	1.31	24.57	0.0263
		0.31	2.37	1.02	12.67	0.0132
	9/6/01	0.68	7.57	2.96	4.82	0.0121
		0.40	3.22	1.58	2.12	0.0095
	7/26/02	0.74	15.31	6.62	18.30	0.0204
		0.37	14.30	4.33	16.07	0.0155
	8/26/03	0.61	6.15	2.69	9.89	0.0149
		0.27	2.45	1.37	5.74	0.0103
Cenaiko	6/26/01	1.05	3.69	1.45	18.22	0.0206
		0.28	3.66	0.75	19.22	0.0233
	7/30/01	1.27	1.80	2.07	11.83	0.0124
		0.23	0.59	0.65	6.31	0.0068
	8/27/01	1.26	1.70	3.92	4.83	0.0058
		0.21	0.60	2.08	0.89	0.0014
	7/1/02	1.42	5.32	2.39	10.85	0.0115
		0.63	4.23	1.63	7.57	0.0080
	8/27/02	1.51	7.83	2.57	4.76	0.0049
		0.24	2.23	1.41	3.80	0.0038
	7/29/03	1.14	2.35	3.54	12.37	0.0135
		0.39	1.06	1.72	8.07	0.0088
Centerville	8/14/02	1.00	13.49	10.20	8.56	0.0142
		0.61	7.42	.	9.67	.

Table 24 Continued

Lake	Date	Density	% Organic	NH_4	KCL ext N	Total Exch N
Cedar	6/19/01	0.60	22.49	3.83	96.36	0.1188
		0.43	16.81	2.14	88.26	0.1178
	8/30/01	0.45	14.92	2.87	23.79	0.0376
		0.40	5.99	0.74	12.57	0.0189
	7/8/02	0.51	30.67	6.11	49.40	0.0611
		0.28	11.62	2.51	28.67	0.0333
	8/8/03	0.23	26.45	5.08	64.62	0.1008
		0.14	14.17	2.62	29.14	0.0504
Gray's Bay	8/6/01	0.11	26.26	5.97	54.43	0.1015
		0.01	4.60	2.22	9.31	0.0243
Harriet	7/2/01	0.94	7.01	3.59	11.65	0.0154
		0.44	3.56	2.31	6.96	0.0094
	9/12/01	0.78	7.29	2.13	12.89	0.0177
		0.44	3.65	1.21	9.06	0.0109
	7/18/02	1.23	6.08	3.28	11.77	0.0136
		0.44	1.08	1.64	16.44	0.0184
	6/16/03	0.49	7.99	4.51	16.51	0.0247
		0.25	3.80	1.87	11.48	0.0164
	8/25/03	0.44	10.78	3.62	21.46	0.0333
		0.32	3.66	1.07	13.84	0.0164
Hiawatha	7/18/02	1.57	3.44	3.55	4.43	0.0046
		0.07	1.87	1.80	2.27	0.0024
	9/12/02	1.55	3.10	.	3.92	0.0052
		0.10	1.19	.	2.76	0.0013
	6/27/03	1.37	1.92	1.63	2.87	0.0029
		0.14	1.05	.	0.62	0.0006
	8/28/03	1.45	1.06	.	3.37	0.0034
		0.05	0.57	.	1.00	0.0010
Isles	6/29/01	0.95	16.78	2.55	32.09	0.0377
		0.49	14.10	1.96	24.87	0.0313
	9/7/01	0.53	27.60	3.42	49.24	0.0793
		0.44	15.76	1.38	33.55	0.0516
	7/9/02	0.60	42.14	2.66	15.58	0.0164
		0.66	55.71	2.03	21.12	0.0221
	8/22/03	0.69	22.65	3.74	51.33	0.0718
		0.44	16.03	1.46	46.01	0.0664

Table 24 Continued

Lake	Date	Density	% Organic	NH_4	KCL ext N	Total Exch N
Otter	6/21/01	0.34	25.25	2.55	177.64	0.1928
		0.20	10.83	1.07	100.28	0.1089
Otter	7/18/01	0.36	27.71	3.64	41.15	0.0546
		0.21	9.70	1.38	20.02	0.0236
Otter	8/28/01	0.35	23.05	2.77	63.58	0.0774
		0.19	8.12	1.13	33.27	0.0439
Otter	6/26/02	0.34	19.50	5.86	60.68	0.0674
		0.20	12.14	4.74	33.36	0.0358
Otter	9/5/02	0.70	40.18	6.92	28.00	0.0319
		0.50	14.08	3.31	23.13	0.0225
Otter	9/18/03	0.15	32.79	4.62	37.70	0.0754
		0.06	6.41	0.84	19.29	0.0365
Shady	8/6/01	0.17	20.21	2.05	26.26	0.0377
		0.04	3.98	1.05	13.84	0.0211
Smith's Bay	6/22/01	0.33	12.52	1.93	24.11	0.0336
		0.19	4.47	0.81	12.52	0.0158
	7/24/01	0.38	13.57	2.42	84.26	0.0973
		0.24	5.15	1.37	62.66	0.0679
	8/23/01	0.37	12.93	3.30	16.02	0.0302
		0.24	4.29	1.16	6.67	0.0136
	7/2/02	0.38	29.00	4.41	39.76	0.0521
		0.12	21.49	1.73	18.54	0.0242
	8/8/02	0.62	17.46	3.48	11.15	0.0155
		0.24	10.55	1.06	5.46	0.0073
Vandalis	8/16/02	1.40	7.54	1.24	2.72	0.0028
		0.23	5.81	.	1.35	.

Analysis across lakes suggests that exchangeable N is not explaining differences in seasonal or yearly average milfoil or total plant biomass. Correlations with mean sample date values (plant biomass and sediment characteristics) for the 10 lakes for which we had sediment exchangeable N and biomass (2001-2003) showed no significant correlation of milfoil average biomass with any sediment parameter (pore water ammonium, bulk density, percent organic, or exchangeable N; all $p > 0.1$ except pore water ammonium). Pore water ammonium was positively correlated with milfoil biomass ($r=0.258$, $p=0.099$) and exchangeable nitrogen was negative correlated, which is contrary to predictions. Mean sediment characters were significantly correlated: bulk density was negatively related to percent organic and total exchangeable N (both $r>0.55$) and ammonium and total exchangeable N were positively related to percent organic. Similar results were found with annual averages except there was no relationship of milfoil and pore water ammonium.

Seasonal and annual average milfoil biomass across the lakes we sampled appears not to be driven by differences in sediment. These results could indicate that our sites, which were all selected for the presence of milfoil varied too little in mean sediment or that other factors such as clarity or herbivores were more important in determining average milfoil biomass during 2001-2003.

We therefore compared plant and sediment characteristics at the sample level (generally 9 samples per lake on each date), first within lakes and then among lakes. Correlations were conducted for plant and sediment variables in each lake for all samples on all dates combined. Relationships among the sediment variables were most consistent. Across all lake analyses, KCL extractable N (does not include pore water) was highly correlated (r typically > 0.95) with total exchangeable N (includes pore water), but pore water ammonium was rarely significantly related to exchangeable N and relationships were positive and negative. Furthermore, exchangeable N was consistently negatively related to bulk density (r typically –0.4 to –0.6) and positively related to organic content (0.3 to 0.5). Thus about 10-40% of variation in exchangeable N can be explained by these variables (which are negatively related). However, bulk density and organics and thus exchangeable N are related to depth and distance from shore, due in part to wave action, scouring and deposition.

Thus several consistent relationships emerged, which inform and constrain interpretation of the influence of sediment: 1) exchangeable N is highly positively correlated with sediment organic matter, and negatively correlated with bulk density, 2) there is no consistent relationship with pore water ammonium (which is more immediately affected by plant density), 3) bulk density decreases with depth (or distance from shore) and organic content increases with depth and 4), exchangeable N is typically lower at the shallowest stations (which also have higher bulk density and lower percent organics) compared to deeper stations.

These relationships can be illustrated more formally with an analysis of sample data from Auburn, Cedar, Otter and Smith's Bay 2001-2003; for these lakes and dates we have complete sediment information (including exchangeable N), depths and plant biomass for 9 sampling sites at each lake on each sampling date. Exchangeable N (mg N/g dry sediment) decreases exponentially with increasing bulk density (Fig. 9; $\ln \text{ExchN} = -4.52 - 1.11 \ln\text{Density(mg/ml)}$; $p < 0.001$, $r^2 = 0.599$) and bulk density explains about 60% of the variation in exchangeable N. Bulk density decreases with distance from shore and depth (Fig. 10: $\ln\text{Density} = -0.67 - 0.74 \ln\text{Depth}$; $p < 0.001$, $r^2 = 0.233$) and thus exchangeable nitrogen increases with depth ($\ln \text{ExchN} = -3.71 + 0.72\ln\text{Depth}$, $p < 0.001$, $r^2 = 0.106$). Although depth only explains about 10% of the variation in exchangeable N, it is a significant factor that should be considered because it will likely also affect species composition and biomass independent of nitrogen.

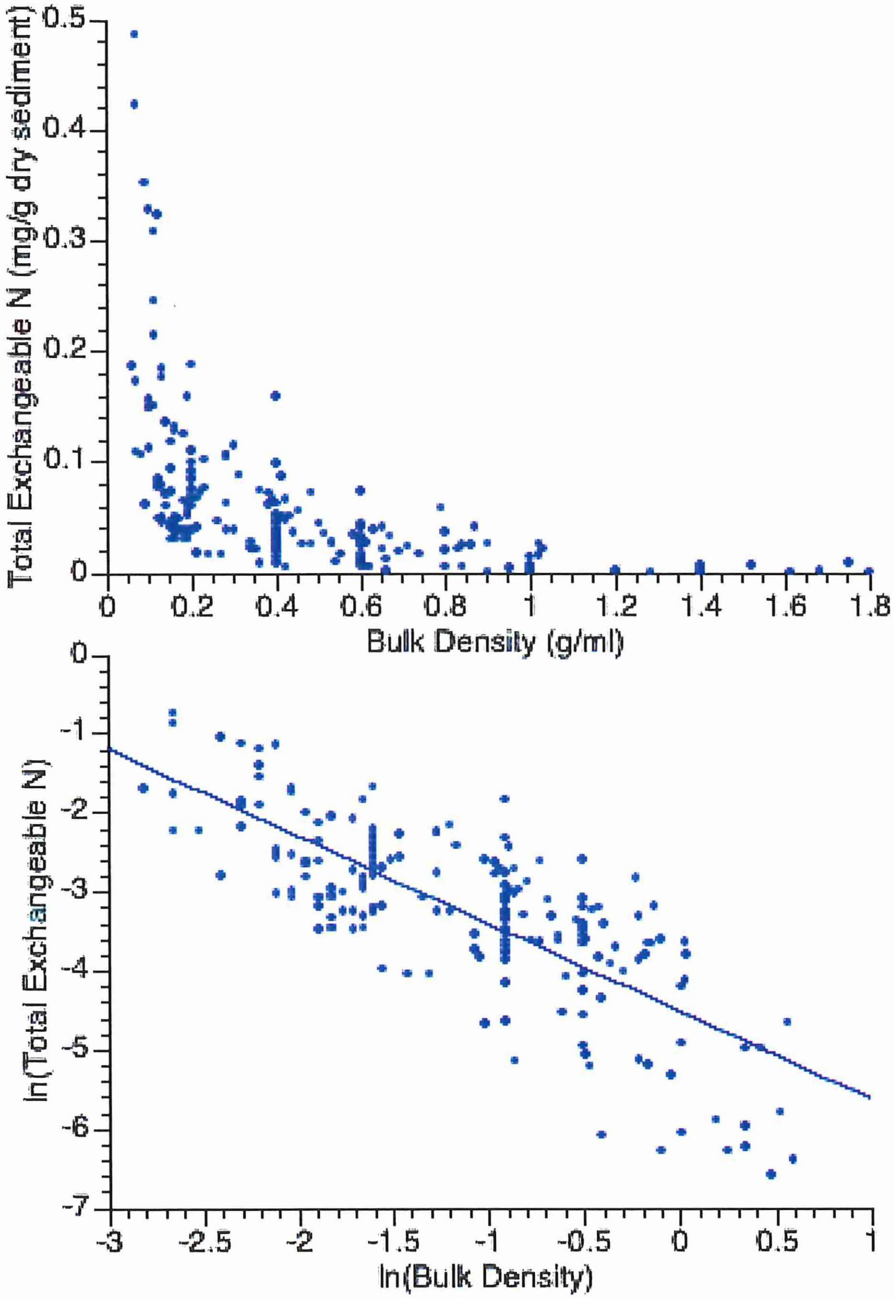

Fig. 9. Relationship of exchangeable N and bulk density from four study lakes. ln ExchN = -4.52 – 1.11 lnDensity(mg/ml); $p < 0.001$, $r^2 = 0.599$

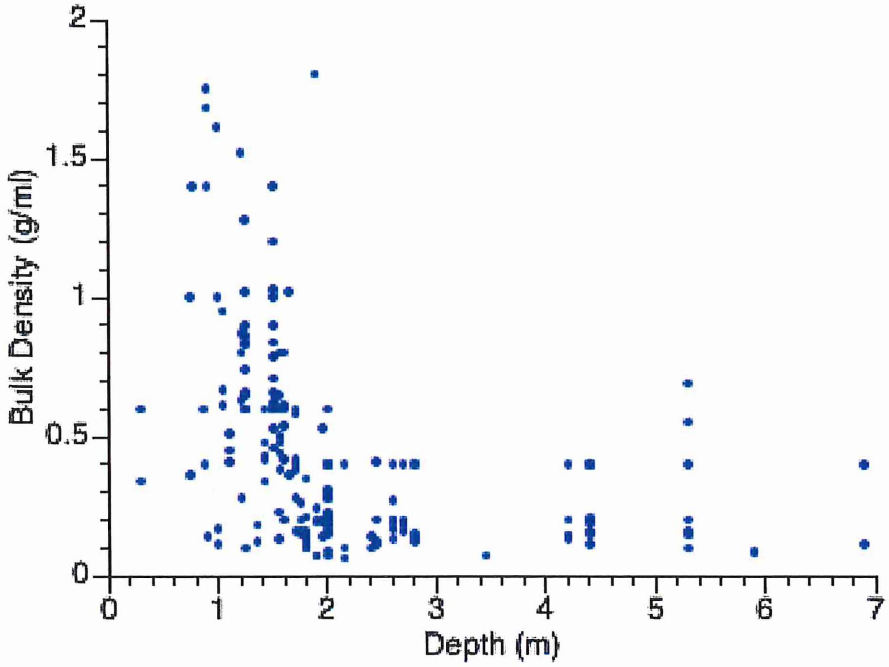

Fig. 10. Bulk density decreases with depth or distance from shore.

Analyses of plant biomass samples collected at the same location as the sediment cores were used to assess the relationship of sediment characteristics to milfoil and native plant biomass. Correlations with the individual samples across the four lakes with depth data (Auburn, Cedar, Otter and Smiths) indicated that milfoil biomass was weakly negatively correlated with bulk density and positively correlated with lnExch N ($p<0.1$) and positively correlated with ln Depth. Milfoil was also negatively correlated with other plants and number of species per sample ($r=-.0237$ and -0.236 respectively, both $p<0.001$). Coontail was also negatively correlated with bulk density, other plants and number of species and positively correlated with depth and total exchangeable nitrogen. Biomass of other plants generally showed the opposite significant relationships (positive correlation with number of species and negative with nitrogen). Pore water ammonium was not correlated with any plant's biomass.

Correlations with the full data set (9 lakes, 370 samples), confirmed some of the above relationships (significance at $p<0.05$). Milfoil biomass was negatively related to bulk density ($r=-0.194$) and positively related to ln ExchN ($r=0.174$) and was negatively correlated with other plant density ($r=-0.148$). Coontail was positively correlated with ln ExchN ($r=0.104$). However, the correlations were generally weaker indicating much variation among lakes.

Correlations were also performed for each lake. Because there were fewer sampling points for each lake (typically 40-50) few correlations with plant variables were significant (although the general relationships among sediment variables were usually significant). Harriet and Auburn showed significant negative correlation of milfoil biomass and bulk density and a positive correlation with exchangeable nitrogen. Calhoun, in contrast, showed a significant positive correlation of milfoil biomass and bulk density and a non-significant negative relationship with total exchangeable N. It is unclear why the plant-sediment relationship in Calhoun was opposite of most other lakes. One possibility is a steeper depth gradient; the shallow sites that supported high biomass of milfoil may have a higher bulk density and the deeper sites with low biomass (due to depth and light limitation) had a low bulk density.

To determine if high milfoil sites within a lake were associated with high exchangeable N or low bulk density we compared means for sites with milfoil biomass > and < 200 g/m^2, High milfoil sites generally had higher exchangeable N and lower bulk density, but the differences were not significant. At Calhoun and Smiths, bulk density and nitrogen were lower at the high milfoil sites, albeit not significantly. We also compared plant biomass at high and low nitrogen sites (> or < 0.01 mgN/g sediment). These comparisons typically showed greater differences, with higher milfoil biomass in the high nitrogen sites. At four lakes, milfoil biomass in high N sites was double that of low N sites, however, the differences were significant only at Smith's Bay and Harriet. Calhoun was again the anomaly with higher (but not significant) milfoil biomass in the low nitrogen sites.

If sediment characters are good predictors of high milfoil biomass, then they should distinguish high and low density milfoil in a discriminant function analysis (DFA). A DFA (Systat 5; Wilkinson 1991) was conducted using the above mentioned individual sample values from the four lakes for depth, bulk density, organic content, pore water ammonium and total exchangeable N to distinguish high (>200 g/m^2) from low (<200 g/m^2) density milfoil sites. None of these variables were significant (multivariate $p > 0.5$, all $p > 0.2$). Further subdividing milfoil biomass into low (<100 g/m^2) medium (100-200) and high (>200 g/m^2) did not result in a significant model. Thus, sediment values alone are not good predictors of high milfoil biomass. If factors such as herbivore damage or water clarity are affecting milfoil density, it may be low at sediment sites where it has the potential to be high. We therefore decided that it might be best to ask if milfoil and other plant community members can discriminate high and low nitrogen sediment sites. A DFA was conducted using milfoil, coontail and other (all other plants) biomass and number of species per sample to discriminate high nitrogen (>0.01 mgN/g sediment exchangeable N) from low nitrogen (<0.01) sites. Milfoil ($p=0.01$), coontail ($p<0.01$) and other plants ($p<0.05$) were all significant as was the overall model ($p =0.001$). Milfoil and coontail showed positive relationships with high nitrogen while other plants were negatively related. Furthermore, the model classified 86% of the 29 low nitrogen sites correctly. It fared more poorly predicting high nitrogen sites; 40% of the high nitrogen sites were classified as low nitrogen sites. However, this misclassification makes sense as these sites likely have high sediment potential but other factors such as herbivores or water clarity reduced milfoil density.

These results suggest that sediment nutrient availability, as reflected in exchangeable N or bulk density do influence milfoil biomass, but at least at the range of values considered in our study lakes, the ability to predict high and low biomass is not strong. Calhoun is a particularly interesting exception, where milfoil biomass on low N sediment (mean of 0.005mg/g) was higher than high N sediment and much higher than milfoil biomass at high N sites in Smith's Bay.

Overall, we found weak support for McComas's hypothesis that exchangeable N can distinguish low milfoil potential sites for high milfoil potential sites. Several confounding factors need to be considered in further analyses. First, if weevils are controlling milfoil then the nuisance milfoil may not exist where it otherwise would. For example, McComas (pers.

com.) determined that nuisance milfoil should occur in most of Otter Lake but did not in 2002, likely due to weevil impacts. Second, shallower sites generally have lower exchangeable N, related to less organics and higher bulk density at these higher energy sites. These shallow sites also tend to have more species and greater abundance of native plants. It is unclear how much of this difference is due to depth vs sediment. Bulk density may be an easier to measure predictor but it also is confounded with depth. Comparisons across similar depths would be most appropriate.

Synthesis:

Four declines of Eurasian watermilfoil in Minnesota appear related to herbivory by biological control agents. Two declines were lake-wide and persisted for 3 or more years. The decline in Cenaiko Lake followed high densities of the milfoil weevil and Eurasian watermilfoil was suppressed for 7 years (<20% of total plant biomass). Native plants became abundant after the decline and a fairly diverse community persisted. Densities of herbivores decreased at Cenaiko after 2001 and by 2003 Eurasian watermilfoil exceeded pre-decline levels and composed 70% of total plant biomass. A decline in Otter Lake was also associated with high densities of the milfoil weevil; milfoil declined from over 350 g dm/m^2 or 80% of total plant biomass in June 2000 to < 10% of plant biomass in 2001 and 26% of plant biomass in 2002. Milfoil increased to 40% of plant biomass in 2003 and it is unclear if the decline will persist. At both lakes, summer average weevil densities exceeded 0.1/stem during and after the decline and often exceeded 0.25/stem.

Milfoil weevils may have suppressed Eurasian watermilfoil at Lake Auburn during several declines. The declines did not persist and macrophytes other than coontail did not become abundant. Milfoil weevils did suppress Eurasian watermilfoil at the shallowest stations in Smith's Bay of Lake Minnetonka; at the shallowest station Eurasian watermilfoil was reduced to <10% of biomass for 8 years and typically <30% of plant biomass at the shallowest two stations (≤ 2.1 m) during this time. Weevil densities at these stations generally exceeded 0.1 per stem and averaged 0.2 per stem over the 8 years. Weevil densities were much lower at deeper stations and did not influence milfoil density.

No declines associated with herbivores were noted at the other lakes we studied. Milfoil remained very dense during the entire 10 yr study period at Cedar Lake and the 5-year study period at Lake Harriet. Weevil and caterpillar densities were quite low at these lakes and although weevils were stocked into both lakes on several occasions, herbivore densities never increased.

Experiments in aquaria, tanks and field mesocosms indicate that milfoil weevils can effectively control Eurasian watermilfoil under controlled conditions; furthermore, numerous field declines of Eurasian watermilfoil have been associated with high densities of milfoil weevils (reviewed by Newman 2004). Our observations and work from elsewhere indicates that milfoil weevils can control Eurasian watermilfoil when adequate weevil densities are reached and sustained. However, in many lakes, weevils do not reach adequate densities or their densities do not persist through the summer over several years to sustain control.

A variety of factors could limit milfoil weevil populations. Work in Minnesota with relatively undeveloped lakes suggests that overwinter conditions are not a major limiting factor (Newman et al. 2001). Low densities of weevils and disappearance of weevils during the summer indicates that in-lake factors are more important at our study sites. Shoreline overwinter habitat may be limiting at some sites and more assessment of shoreline habitat is needed. Jester et al. (2000) and Tamayo (2003) found that weevil densities were higher in lakes and areas with less undisturbed shoreline and high levels of development or winter shoreline flooding may limit overwinter habitat and survival. Parasites and pathogens also do not appear to be important (Newman et al. 2001), although more investigation is warranted.

Predation by fish, particularly sunfish, does appear to be an important limiting factor. Sutter and Newman (1997) showed that sunfish prey on milfoil weevils (primarily adults) and a high density of sunfish could theoretically limit weevil populations. Ward (2002) showed that adult (female) longevity is critical to developing high weevil populations. Because the milfoil weevil is iteroparous (and can live for several months), laying several eggs per day, female egg laying longevity is very important; doubling female egg laying longevity from 3 to 6 days can result in an 8-fold increase in late summer weevil populations. Fish predation on adults would reduce female longevity and can therefore have a large effect on end of summer population density.

Stocking and cage experiments at Cedar and Otter Lake indicate that sunfish can reduce herbivore establishment and density (Newman et al. 2002, Ward 2002). Our surveys of weevil density compared to sunfish density further indicate that sunfish are limiting weevil densities in many of our lakes. Over 70% of variation in adult weevil density was explained by sunfish trapnet catch and total weevil density appears to respond to a threshold of sunfish density. At sunfish densities < 30/trapnet weevil densities have a high probability of exceeding 0.3/stem (adequate to control milfoil) but at greater sunfish densities, weevil densities are <0.1 per stem. Sunfish > 6cm (age II or older) can prey on adult weevils (Ward 2002) and it is likely that abundant small sunfish that use vegetation are the major source of mortality. Both sustained declines in Minnesota occurred with low sunfish populations and the decline of weevils and loss of milfoil control at Cenaiko when sunfish increased to 25/trapnet further indicates that low sunfish densities may be required for successful control.

Work from elsewhere is also indicating that fish predation may be an important limiting factor. In New York, Lord and Johnson (see Lord 2003) have shown that sunfish may be limiting *Acentria* and weevil populations. Parsons et al. (2003 and J. Parsons, personal communication) also have evidence that sunfish are limiting weevil populations and ability to control milfoil in Washington state. Furthermore, the oft-cited weevil induced decline at McCullom Lake, IL (see Creed 1998) occurred the summer following a rotenone treatment that eliminated all fish (R.L. Kirchner, personal communication). Brownington Pond, the site of one of the best-documented declines caused by the milfoil weevil (Creed and Sheldon 1995, Sheldon and Creed 1995), lacks sunfish, and perch, which are present, do not appear to consume milfoil weevils. Thus an increasing body of evidence suggests that high sunfish populations will limit control agents including the milfoil weevil.

The distribution of milfoil weevils within lakes also suggests that fish predation may be important. Weevils appear to do better in large shallow expanses of milfoil rather than steeper areas that may provide better access by fish (Newman 2004). Tamayo et al. (2000) and Jester et al. (2000) found higher densities of weevils in shallow sites and Lillie (2000) found highest densities of weevils in shallow and moderate depths and much lower densities at the deep edges. In Minnesota we also find the highest densities of weevils at shallow to moderate depths (<3m; see above and Newman et al. 2002). Johnson et al. (2000) found weevil densities negatively correlated with lake depth and suggested weevils do better in shallow lakes. These relationships do not appear to be related to distance from shore (Jester et al. 2000, Newman 2004) but are more likely related to depth. Deeper plants likely allow more ready access to predation by fish and wave action might also limit weevils by disrupting adults or breaking plant parts inhabited by larvae or pupae.

There may, however, be a negative feedback of high plant density in shallow sites. High plant density may favor development of large populations of small sunfish (e.g., Olson et al. 1998), which could then limit milfoil herbivore populations, promoting denser plants, and more abundant small sunfish. Once an abundant population of small sunfish develops, it may be difficult to shift the sunfish population and develop significant herbivore populations.

Stocking or augmenting weevils will likely be ineffective in lakes with high sunfish densities. Previous open augmentations in Cedar and Isles in 1996 proved to be ineffective (Newman et al. 1997b) and did not establish weevil populations. Stocking into cages at Cedar Lake did establish populations within sunfish exclusion cages, but despite the stocking of several thousand weevils into open and closed cages at Cedar each year from 1998-2001, a viable weevil population has not developed at Cedar Lake. Stocking of higher densities of weevils in open plots at Lake Harriet in 2002 and 2003 may have resulted in establishment of a weevil population, however, by end of summer the densities were very low and the population was too low (0.04/stem summer average in 2003) to have any effect on milfoil. All of these lakes have high sunfish densities (>100/trapnet).

Weevil stocking may have been more successful in Hiawatha, a low sunfish (11/trapnet) lake, however, weevil densities were not adequate to cause an obvious milfoil decline at Hiawatha. Weevils did overwinter at Hiawatha and in 2003 the summer mean density was 0.12/stem. Additional monitoring should be done to determine if weevil populations will increase at Hiawatha. It is possible that several years may be required to develop populations adequate for control, however, population modeling suggests that populations should develop quickly if female survival is high (Ward 2002).

Biocontrol of milfoil will likely be effective only in lakes with low sunfish density and because milfoil weevils and other herbivores (*Acentria* and *Parapoynx*) appear widespread, natural populations may develop in these lakes, obviating the need for stocking. Sunfish populations do appear variable (e.g., Cenaiko Lake, Shroyer et al. 2003) and stocking or augmentation might be viable in situations where sunfish have been controlled or are not present.

Reducing overabundant sunfish populations should be explored as one approach to enhance control; in addition to enhancing milfoil biocontrol, better size structured (i.e., low density of large fish) sunfish populations are desired by fisheries manager (e.g., Cross et al. 1992, Olson et al. 1998, Jacobson *in press*). Reducing overabundant sunfish is not trivial and enhancing predators (e.g. Shroyer et al. 2003) or manipulating macrophytes (e.g., Cross et al. 1992, Olson et al. 1998) alone is likely to not be successful and angling restrictions on sunfish may also be required (e.g., Jacobson *in press*). Experimental management to reduce overabundant sunfish populations to enhance herbivores and biological control should be considered. It is likely that a combination of sunfish regulations (reduced creel limits for larger fish), enhancement of predator populations and vegetation manipulation (e.g., strip cutting) might be required to shift sunfish populations to a less abundant and more balanced size structures. It is interesting to note that the milfoil decline in Fish Lake, WI (Lillie 2000) occurred during an assessment of strip cutting to enhance sunfish and bass populations (Olson et al. 1998, Unmuth et al. 1999), however, it appears that the decline occurred prior to and during the manipulation and that weevil densities declined the year after the strip cutting. The increased edge may have simply increased sunfish access to milfoil weevils and the effects of plant manipulations will need to be carefully considered to achieve the desired results.

A positive native plant response is important to the sustained biological control of invasive weeds (Newman et al. 1998). In the two lakes where declines persisted (Cenaiko and Otter), an array of rooted native plants responded positively and developed substantial biomass. Similarly, at the shallow stations in Smiths Bay, rooted native plants replaced the Eurasian watermilfoil. Conversely, at Lake Auburn, rooted plants did not appreciably increase and coontail remained the dominant native plant. It should be noted that during the last two years of the decline at Cenaiko, rooted plants became less common and coontail was the dominant native plant. Because coontail is not rooted, it may be less able to displace milfoil, however, it may also be better adapted to coexist with milfoil. In many of the lakes with high milfoil biomass, coontail is the second most abundant plant. The general lack of negative correlations between coontail and milfoil, despite their being the dominant plants in most of the study lakes, suggests they are readily able to coexist and there may be some yet undetermined facilitation between these plants.

Our removal experiments shed some light on these interactions but suggest that a positive rooted plant response may not be expected in milfoil-coontail dominated systems. In the lower water clarity, milfoil-coontail community at Lake Auburn, coontail quickly filled in when milfoil was removed but was eventually replaced by milfoil. Milfoil maintained dominance in the controls or when native plants were removed but rooted native plants did not respond positively when milfoil or all plants were removed. A similar response was seen in the higher clarity milfoil-coontail community at Cedar Lake. Coontail was able to colonize removal plots within the first season, but by the second year milfoil returned to pre-removal levels and rooted natives did not respond. It does not appear that clarity alone was inhibiting the colonization by rooted plants, although the response to removals at different times of the year may be different. In contrast, at Otter Lake, where herbivory was important during the manipulations, Eurasian watermilfoil was suppressed by herbivores and did not respond to the manipulations. Coontail was able to initially respond to removals but as the summer progressed rooted plants had responded positively and by the second year were dominant. With herbivore pressure and a positive rooted plant response a more desirable community was maintained. Unfortunately in all three experiments, the communities returned relatively quickly to the control situation – either milfoil-coontail or more diverse rooted plants. It is not clear if the failure of rooted plants to respond at Cedar and Auburn was due to lack of propagules or some direct suppression by milfoil or coontail.

Attempts to increase water clarity via alum treatments also did not enhance native plant communities. In the three Minneapolis lakes with successful alum treatments, Eurasian water milfoil maintained or increased its dominance after alum treatments. It is possible that the improvements in clarity were not sufficiently large or sustained for a long enough time to benefit native plants. Alternatively, a milfoil stressor, such as herbivory, may be needed to reduce milfoil's competitive advantage and dominance. The Minneapolis lakes have very high sunfish densities and very low herbivore populations.

It is likely that recovery of rooted native vegetation will be important for successful chemical control as well as biological control. More work to enhance positive native plant response after milfoil control would be very useful.

McComas (1999, 2003) proposed that sediment nitrogen may be a good predictor of nuisance levels of Eurasian watermilfoil; high nitrogen sites (> 6ppm exchangeable N expressed per volume) should support dense growths of milfoil while lower nitrogen sites would not support nuisance levels of milfoil and would be more amenable to native plants that are adapted to lower nitrogen levels. At low nitrogen sites, Eurasian watermilfoil should not reach nuisance levels. We found weak support for McComas's hypothesis and the confounding effects of depth, bulk density and exchangeable nitrogen should be considered in any analysis. Bulk density decreases with depth and exchangeable N is negatively correlated with bulk density; thus shallow sites tend to have lower exchangeable N.

Milfoil biomass across lakes was positively correlated with exchangeable N, however the relationship was weak (explains < 4% of variation in milfoil biomass). Sediment characters were not able to discriminate high and low density milfoil sites, likely because other factor such as herbivory and water clarity were more important determinants of low milfoil biomass. Plant biomass was however able to discriminate high (>0.01 mgN/g sediment exchangeable N) from low nitrogen (<0.01 mgN/g) sites and 86% of low nitrogen sites were correctly classified (but many high nitrogen sites were incorrectly classified as low nitrogen). Furthermore, the classification indicated that milfoil and coontail were positively associated with high nitrogen and other plants negatively loaded with high nitrogen, as McComas predicted. Most of our sites have higher nitrogen than the level that might limit milfoil growth and it is unclear if calculating nitrogen on a volume basis rather than a dry mass basis (standard aquatic protocol) would affect the results. Thus sites with low exchangeable N (<0.01 or 0.001 mg N/g) might on average be expected to support lower biomass of milfoil but the predictions are not precise. Biomass at Calhoun on sediments with <0.005 mgN/g occasionally exceeded 200 and in several cases 400 g dm/m^2.

Initially we speculated that poor sediment conditions at Cenaiko Lake may have facilitated the milfoil decline and that higher weevil densities might be required to facilitate declines on more fertile sediments (Newman and Biesboer 2000). The decline at Otter suggests this is not the case as the decline there occurred with lower weevil densities and much "better" sediment (higher organics, lower bulk density and higher exchangeable N). Thus the two lakes with clear milfoil declines, Otter and Cenaiko, represent opposite ends of sediment fertility, suggesting that herbivore induced declines are not limited to poor or highly fertile sites.

In summary, the milfoil weevil can cause sustained declines of Eurasian watermilfoil if sufficient densities are maintained throughout the summer each year. Sunfish appear to be limiting herbivore densities in many lakes and lakes with high densities of sunfish will likely not support adequate weevil populations to achieve milfoil control. A positive rooted native plant response is also likely required for sustained control and more research into methods to reduce sunfish predation and to enhance native plant response is needed.

<u>Conclusions</u>

- Sustained milfoil declines associated with the milfoil weevil occurred in two lakes. The decline at Cenaiko Lake persisted for 7 years and at Otter Lake for three years. Milfoil was also suppressed for more than 7 years at the shallowest (≤2m) sites at Smith's Bay of Lake Minnetonka, but not at deeper sites. Limited and variable control was seen at Lake Auburn.

- Adequate weevil densities that persist throughout the summer are required for sustained milfoil declines. Lakes with low densities of weevils (<0.1 per stem) showed no evidence of herbivore induced declines during the 5-10 years of study (Cedar, Isles, Calhoun, Harriet).

- Weevil densities appear limited by sunfish predation. Lakes with persistent declines had low densities of sunfish and when sunfish densities increased at Cenaiko Lake to 25/trapnet, the weevil population was greatly reduced.

- Comparison of milfoil weevil densities in 11 lakes with sunfish densities determined by DNR Fisheries assessments shows that weevil density declines significantly with increasing sunfish density. Sunfish densities greater than 25-30 per trapnet may severely limit weevil populations and their ability to control Eurasian watermilfoil. These results confirm that fish predation is an important limiting factor in Minnesota lakes.

- Augmentation or stocking of weevils into high sunfish density Lake Harriet resulted in establishment of a weevil population but the densities were low and may not persist. Densities of herbivores were too low to have a significant effect on milfoil biomass. Stocking weevils into a low fish density lake (Hiawatha) resulted in establishment of weevils and the population appeared to be increasing after the second year of stocking. Weevil populations, however, did not build to high densities predicted by modeling. A significant decline of milfoil due to herbivores was not found, but herbivores may have limited the expansion of milfoil at Hiawatha. Future stocking or augmentation should not be conducted in high sunfish density lakes.

- Plant community manipulation experiments in high and low clarity milfoil-coontail lakes showed that coontail can colonize quickly when all plants or milfoil are removed but within a year milfoil will return to dominance. Rooted plants did not become abundant and milfoil and coontail remain dominant where not controlled by the milfoil weevil. At sites where milfoil is controlled by herbivores, coontail can initially be successful but rooted plants can dominate over the summer and in following years. More work on reestablishing rooted plants communities after control of Eurasian watermilfoil is needed.

- There is some support for McComas's hypothesis that native plants will do better on low nitrogen sites and milfoil biomass will not reach nuisance levels on low nitrogen sites but milfoil will reach nuisance levels on high nitrogen sites. If milfoil is controlled by factors other than sediment, such as herbivory or water clarity, it will not reach nuisance levels. High levels of milfoil biomass appear less common on low nitrogen sediments and low and high nitrogen sediments can be discriminated by milfoil and native plant biomass but exceptions were found.

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Appendix I. Key to plant abbreviations used in this report.

CHA	*Chara* spp. (muskgrass)
CRT	*Ceratophyllum demersum* (coontail)
ELD	*Elodea canadensis* (Canada waterweed)
HET	*Heteranthera dubia* (mud plantain) = *Zosterella dubia* (ZOS)
LMR	*Lemna minor* (lesser duckweed)
LTR	*Lemna trisulca* (star duckweed)
MGD	*Megalodonta beckii* (water marigold)
MSI	*Myriophyllum sibiricum* (northern watermilfoil)
MSP	*Myriophyllum spicatum* (Eurasian watermilfoil)
NAJ	*Najas* spp.
NMP	*Nymphaea* spp.
NUP	*Nuphar* spp.
PAM	*Potamogeton amplifolius* (largeleaf pondweed)
PBE	*Potamogeton berchtoldi* (Berchtolds' pondweed)
PCR	*Potamogeton crispus* (curled pondweed)
PDI	*Potamogeton diversifolius*
PEC	*Potamogeton pectinatus* (sage pondweed) (now *Stuckenia pectinata*)
PFO	*Potamogeton foliosus* (leafy pondweed)
PGR	*Potamogeton gramineus* (variable pondweed)
PIL	*Potamogeton illinoensis* (Illinois pondweed)
PNA	*Potamogeton natans*(floating leaf pondweed)
PNO	*Potamogeton nodosus* (river pondweed)
PRI	*Potamogeton richardsonii* (claspingleaf pondweed)
PRO	*Potamogeton robbinsii* (Robins' pondweed)
PSP	*Potamogeton spirillus* (snailedseed pondweed)
PZS	*Potamogeton zosteriformis* (flatstem pondweed)
RAN	*Ranunculus* spp. (white water buttercup)
SPO	*Spirodela polyrhiza* (greater duckweed)
VAL	*Vallisneria americana* (wild celery)
UTV	*Utricularia vulgaris* (bladderwort)

Final Report for Result 2, Activity 1
(2001 LCMR- Biological Control of Eurasian Watermilfoil and Purple Loosestrife-Continuation)

Population Dynamics and Long-term Effects of *Galerucella* spp. on Purple loosestrife, *Lythrum salicaria,* and non-target native plant communities in Minnesota.

BY

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Abstract
A field study was conducted to assess population dynamics and long-term effects of the biological control agent *Galerucella* spp. on Purple loosestrife, *Lythrum salicaria,* and non-target native plant communities in Minnesota. Five *Galerucella* spp. release sites in central and southern Minnesota were studied between 1995 and 2003. *Galerucella* spp. established at all five release sites following additional release of insects at three locations. At all five release locations, *Galerucella* spp. populations peaked between three and five years after successfully establishing. As a result, purple loosestrife densities, height and flowering were reduced across all sites. After the initial peak in *Galerucella* spp. densities, all sites saw a decline of *Galerucella* spp. abundance in response to the reduction in purple loosestrife abundance. *Galerucella* spp. and loosestrife abundance followed two distinct patterns over time. The *Galerucella* spp. populations either rebounded with increasing loosestrife abundance or the *Galerucella* spp. population did not rebound. Our results suggest that *Galerucella* spp. can provide effective control of purple loosestrife and increase plant species richness. However, there may be limitations whereby some insect populations decline precipitously after reaching high densities and do not recover following declines or have not been observed to recover in the time frame of this study. Continued monitoring will be needed on those sites thatn did not rebound to determine if the *Galerucella* spp. populations will once again increase and control the purple loosestrife without reintroducing the beetles.

1. Introduction

One of the major criticisms of weed biological control of weeds is the lack of post-release studies that document the long-term effects of the introduced agents (Blossey and Skinner 2000, McClay 1995, McEvoy and Coombs 1999). Most post-release monitoring efforts have focused on agent establishment and spread with little quantitative data on host suppression (Crawley 1989, McClay 1995). In particular, there is a need to document control agent populations over time and effects on the target pest plant and associated plant communities. Such studies can provide knowledge of success or failure of a biological control effort but also provide insight to predict outcomes of future biological control programs better (Blossey and Skinner 2000, McFayden 1998, McEvoy and Coombs 1999). Classical biological control of purple loosestrife, *Lythrum salicaria* L., in North America provides an opportunity to develop long-term studies on the impact of release biological control agents.

Purple loosestrife, *Lythrum salicaria* L., is a perennial emergent wetland plant introduced into North America from Europe (Stuckey 1980, Thompson et al. 1987). Since its introduction, purple loosestrife has become established across the northern half of the United States and Canada (Stuckey 1980). Purple loosestrife is a herbaceous perennial which forms a woody crown from which new shoots emerge every year (Shamsi and Whitehead 1973). Seed dispersal, rather than vegetative reproduction is the major means of dissemination. It is estimated that each plant is capable of producing up to 2.7 million seeds per season (Thompson et al. 1987). The prolific seed production and subsequent seed rain leads to the creation of an extensive seed bank (Welling and Becker 1990). Once a seedbank is established, purple loosestrife more successfully colonizes disturbed and open sites than do native species (Thompson et al, 1987, Welling and Becker 1993).

Invasions by purple loosestrife have been associated with ecosystem impacts including reduction of native plant diversity and abundance, reduction in wildlife habitat, and changes to wetland function as described by Blossey et al. (2001a). In particular, there are numerous studies where purple loosestrife has been shown to be highly competitive compared with other native wetland species (Gaudet and Keddy 1988, Gaudet and Keddy 1995, Mal et al. 1997, Rawinski and Malecki 1984, Weiher et al. 1996, Weihe and Neely 1997, Welling and Becker 1990).

Efforts to manage purple loosestrife with conventional control methods such as chemical application, cultural practices and mechanical removal, provide only limited, short-term control and are only effective on small populations (Blossey et al. 2001b, Skinner et al. 1994, Welling and Becker 1993, Welling and Becker 1990). Experience in Minnesota suggests that controlling large, established populations of purple loosestrife with conventional methods is rarely successful because of the large seedbank allows the population to rebound following control (Skinner et al. 1994, Welling 1990).

Classical biological control is considered an alternative to conventional control methods and may provide long-term control of purple loosestrife (Blossey et al. 1996, Malecki et al. 1993). In 1992, *Galerucella calmariensis* L. and *G. pusilla* Duft. (Coleoptera: Chrysomelidae) were introduced to control purple loosestrife in North America and have become established across the north temperate portion of the United States and Canada (Hight et al. 1995, Lindgren et al. 2002). Since 1992, there have been a number of reports documenting the establishment, control success and non-target impacts caused by *Galerucella* spp. (Blossey 1995, Blossey et al. 2001a, Blossey et al. 2001b, Blossey and Skinner 2000, Corrigan et al. 1998, Dech and Nosko

2001, Katovich et al. 1999, Katovich et al. 2001, Kaufman and Landis 2000, Landis et al. 2003, Lindgren 2000, Lindgren 2003).

In Minnesota, *Galerucella* spp. were first released for the biological control of purple loosestrife in 1992. Since then, more than eight million beetles have been released on more than 800 purple loosestrife infestations statewide. To effectively evaluate the biological control program within Minnesota, long-term monitoring was initiated. The objectives of our studies were to quantitatively assess the population dynamics of *Galerucella* spp. as well as document their impacts on purple loosestrife and associated wetland plant species for up to nine years post-release at multiple sites.

2. Materials and Methods

2.1. Study sites and *Galerucella* spp. releases

Five study sites were chosen in central and southern Minnesota based primarily on their histories of having the earliest releases of *Galerucella* spp. in the state. The sites are located near the following cities or lakes: Winona, Reno, Circle Lake, White Bear Lake and Big Marine Lake.

Winona, MN. The Winona site is a 3.2 ha palustrine wetland located in southeastern Minnesota near the Mississippi river in Winona County and within the city limits of Winona (Table1). Although the wetland is near the Mississippi river, the wetland is recharged by overland flow from nearby blufflands and runoff from impervious surfaces (roads and parking lots). For much of the year, a portion of the Winona wetland has standing water, while the edges tend to have saturated soils. The Winona wetland vegetation community had been dominated by purple loosestrife for more than 20 years and at the time of release was essentially a monoculture of purple loosestrife covering 95% of the wetland with only a few native plants found around the margins of the wetland. The plant community other than loosestrife consisted of cattails, *Typha* spp., rushes, *Scirpus* spp., a variety of sedges, *Carex* spp., and grasses, *Gramniae* spp. *Galerucella calmariensis* and *G. pusilla* were first introduced in 1993 when 1,000 adults were released directly on to loosestrife plants. The insects released were collected immediately prior to their release from Germany where they were field collected and shipped to Minnesota. The insects were a mixture of the two species, however, there was no determination of the percentage of each. Visual surveys carried out in 1994 and 1995 found little evidence of *Galerucella* spp. establishment with only a few adults and egg masses found each year. Consequently, more than 4,000 *Galerucella* spp. were released in 1995 and 6750 in 1996, in an effort to establish the control agents in this wetland (Table 1). The 1995 and 1996 releases were made from colonies reared on loosestrife plants in cages out doors and in the greenhouse at the University of Minnesota as described by Loos and Ragsdale (1998).

Reno, MN. The Reno site is an 11 hectare palustrine wetland located in Houston County near the border with Iowa and Wisconsin about 2.5 miles south of the city of Reno, MN (Table1) This wetland is a backwater area of the Mississippi river and is prone to seasonal flooding. The Reno site had also been dominated by loosestrife for more than two decades. Associated plant species were cattail, bur-reed, *Sparganium eurycarpum*, and bulrush, *Scirpus validus*. One thousand adult *Galerucella* spp. were released directly on to purple loosestrife plants in 1993. As in the Winona site, the insects were part of the same collection from Germany. The *Galerucella* spp. failed to establish two years after release, therefore an additional 4000 *Galerucella* spp. were released at the same location in 1995 from adults reared on plants in a greenhouse during late winter and early spring. The subsequent releases were made by adult beetles were released by placing fine meshed sleeve cages over purple loosestrife plants and then placing 200 to 300 beetles within each cage (referred to as the sleeve cage method). The sleeve cages were removed one week later after egg deposition had occurred.

White Bear Lake, MN. The White Bear Lake location is a 13.8 hectare wetland in Ramsey County in east-central Minnesota within the city limits of White Bear Lake (Table 1). This is a shallow wetland that is seasonally flooded and largely dominated by cattail, except for the southern one third of the wetland, which is dominated by purple loosestrife. In 1993, this site received its first release of 1,000 adult *Galerucella* spp. from same collection and shipment from Germany as the Winona and Reno sites. Similar to the Winona and Reno sites, only a few egg masses and adult *Galerucella* spp. were observed in 1994 and no evidence of beetles were observed in 1995. Consequently, more than 4,000 laboratory and greenhouse reared *Galerucella* spp. were released in 1995 using the sleeve cage method.

Circle Lake, MN. The Circle Lake site is a 25 ha palustrine wetland located along the lakeshore of Circle Lake in Rice county (Table 1). This shallow marsh is semi-permanently flooded with a gradient from saturated soils to standing water. The wetland is approximately 50-200 meters wide ringing two thirds of the lake edge. The vegetation was 50% dominated by purple loosestrife with a diversity of native plants such as sedges, *Carex* spp., river bulrush, *Scirpus fluviatilis,* and smartweeds, *Polygonum* spp., at the drier edge and cattail and bur-reed at the wet edge adjacent to the lake itself. The loosestrife had been established at the site for over 20 years and had spread throughout the wetland complex. 500 greenhouse reared *Galerucella* spp. were released in 1994 using the sleeve cage method.

Big Marine Lake, MN. The Big Marine Lake site is a 26 ha palustrine emergent shoreline located in Washington County in east-central Minnesota. This site is a wet meadow that has saturated soils and predominant vegetation type is sedges and grasses. Purple loosestrife was found throughout the wet meadow with areas where purple loosestrife was the dominant plant. This site, however, is not considered to have a monoculture of purple loosestrife. Adjacent to this wet meadow is another large wet meadow 40 hectares in size that was dominated by purple loosestrife. The first release at this location was in 1998. The *Galerucella* spp. for their release were captured earlier in the year from the Circle Lake site and placed on potted plants inside a sleeve cage. The *Galerucella* spp. reproduced within the cage and approximately 7,000 F_1 offspring were released by placing potted purple loosestrife plants with larvae, pupae, and new emerged adults next to purple loosestrife plants at this site. An additional estimated 21,000 beetles were released in 1999 using the same release technique.

2.2. Sampling design

To monitor changes in insect and plant communities over time within each site, we adapted the standardized monitoring protocol described by Blossey and Skinner (2000). Transects, 50m to 75m in length, were established at each field site. Permanent 1m^2 quadrats were placed every 12.5 meters along each transect. The corners of each quadrat were marked with posts. Six transects with a total of 30 quadrats were established at the Circle Lake site in 1995 (Table 2). Four transects were place near the original release point, while two transect were placed 400m away to serve as controls. In 1997, four additional sites including Big Marine Lake, Reno, White Bear Lake and Winona, were established with two transects each at least 50m apart near the initial release point. Five to seven permanent quadrats were established on each transect for a total of 11to 14 quadrats at each site (Table 2).

2.3. Sampling *Galerucella* spp., purple loosestrife and other vegetation

At each location, the quadrats were non-destructively sampled twice each year. Sampling occurred once in the spring to capture *Galerucella* spp. abundance and once in late summer to capture impacts to purple loosestrife and abundance of other plant species present. In the spring (late-May to early-June), we timed our sampling to coincide with the phenology of purple loosestrife plants. Sites were sampled when the majority of the loosestrife plants ranged from one to three feet in height. Due to a faster accumulation of growing degree-days at southern latitudes, sites were surveyed from south to north over a three week period. This was to ensure *Galerucella* spp. presence and oviposition was occurring and could be quantitated at each site. At each location, each quadrat was sampled for the number of *Galerucella* spp. egg masses, larvae and adults. This was carried out by visually counting each insect life stage separately. We counted the adults first as they were likely to drop off the plants if disturbed, and then counted the number of larvae and egg masses. In 2004, 200 *Galerucella* spp. were collected from four of the five sites for species identification. The first 100 male beetles were dissected and identified using morphological characteristics of the adeagus to provide a ratio of each species present.

Quadrats were revisited in late summer (late August) to record purple loosestrife percent cover, number of stems, height (five tallest plants) and the total number of inflorescences. In addition, the percent cover for each species present other than purple loosestrife was visually estimated. Sites were revisited each year for up to 9 years after release.

2.4. Data Analysis

Due to the variability of the sites and insect releases, we chose to analyze each site separately and standardize for number of years after *Galerucella* spp. introduction. Each quadrat was treated as a replicate in a completely randomized design. The number of quadrats (replicates) for each site is found in Table 2. For each site, the mean ± SE of *Galerucella* spp. egg mass density, purple loosestrife stem density, percent visual cover, total number of inflorescence, stem height and number of plant species other than purple loosestrife, were calculated for the number of years after initial release. We chose to use egg mass density as our indicator of *Galerucella* spp. abundance because the adults tend to aggregate and move readily with in a site and larvae can be hidden in the apical meristems of the plant. Egg masses are easily observed; they are stationary and remain on the plants for up to two weeks, providing a manageable timeframe in which to conduct the surveys. Analysis of variance (ANOVA) and Ryan-Einot-Gabriel-Welsch Multiple Range Test (PROC GLM, SAS Institute 2001) were used to analyze differences among the number of years after release for density of *Galerucella* spp., purple loosestrife variables, and number of species other than purple loosestrife observed.

3. Results

Galerucella spp. established at all five release sites following additional release of insects at three locations. *Galerucella* spp. did not establish after the initial release of adults in late summer at Reno, White Bear Lake and Winona. The initial releases at these three sites were from *Galerucella* spp. collected in Europe and shipped to Minnesota in July of 1993. After two years of finding very little evidence of establishment at Reno and Winona, and no evidence of *Galerucella* spp. at White Bear Lake, additional releases were made with adults reared in outdoor cages on potted plants, with the potted plants containing primarily pupae and adults of the F_1 generation.

Circle Lake was the first site in Minnesota where *Galerucella* spp. became established. This initial release in1994 was made using sleeve cages on purple loosestrife plants to confine the beetles, with the hope that mating and egg laying would occur before cages were removed and insects could disperse. One week after initial release, sleeve cages were removed and we observed mating pairs and high numbers of egg masses of *Galerucella* spp. on each plant. In subsequent observations during the year of release, we observed hundreds of larvae that eventually defoliated the purple loosestrife plants on which the beetles were initially placed. One year after release, *Galerucella* spp. were observed scattered up to 100 meters from the original release point. *Galerucella* spp. egg mass densities fluctuated significantly (F= 7.38, df= 6,197; *P*= <0.0001) over time. Mean number of egg masses per m^2 ranged from a high of 22.7±4.6 four years after release to a low of 1.1±0.3 nine years after release (Figure 1a). As the *Galerucella* spp. densities peaked, the first impacts were a reduction in purple loosestrife height and number of inflorescences. (Figure 1c-d). This was followed by reduction in stem densities (Figure 1b). *Galerucella* spp. populations cycled from high to low densities over the 9-year period with a second peak density measured seven years after the initial release. Even with the population fluctuations, purple loosestrife stem density, height and flowering did not rebound (Figure 1b-d). In particular, stem density steadily declined and total number of inflorescence remained near zero for the past six years. The number of species other than purple loosestrife changed over time (F= 6.52, df= 6, 202; *P*= <0.0001). Six years after release the number of species other than purple loosestrife peaked at 3.8 ± 0.3 species per m^2 compared to low of 2.2 ± 0.2 three years after release (Figure 1e). Outside the study plots, we observed *Galerucella* spp. up to 1.5 km from the release point four years following the initial release.

In Winona, *Galerucella* spp. became established and egg mass densities remained above 20 egg mass per m^2 for the first three years after additional releases were made in 1995 and 1996 (Figure 2a). A subsequent reduction in purple loosestrife flowering, and stem height, followed by a reduction in stem density occurred by three years after release (Figure 2b-c). As the purple loosestrife stem density was reduced to near zero four years after release, egg mass density declined sharply (Figure 2a-b). The lack of purple loosestrife continued to cause a decline in *Galerucella* spp. egg mass density five years after release. With a lack of insect pressure, the purple loosestrife rebounded in stem density and stem height, while flowering continued to be suppressed. *Galerucella* spp. responded to the purple loosestrife increase with a spike in egg mass densities (Figure 2a), which in turn, was followed by a reduction in purple loosestrife stem density and height (Figure 2b-c). Over the seven-year period, the number of plant species other than purple loosestrife increased (F= 10.78, df= 6,84; *P*= <0.0001). The number of species other than purple loosestrife increased from a low of 0.4±0.2 one year after release to a high of 2.4 ± 0.3 species per m^2 four years after release (Figure 2b,e).

Egg mass densities fluctuated dramatically at White Bear Lake (F= 11.47, df= 6, 70; *P*= <0.0001)(Figure 3a) and Reno (F= 11.41, df= 6, 73; *P*= <0.0001)(Figure 4a) over time. Both sites followed similar patterns with *Galerucella* spp. populations peaking five years after release then collapsing to near zero by eight years after release (Figures 3a and 4a). Egg masses per m^2 reached a peak of 80.5 ± 20.9 at White Bear Lake and 85.8 ± 22.4 at Reno, which were four times higher than the intial peak following release at Circle Lake or Winona. There was a corresponding decrease in purple loosestrife height (F= 31.3, df= 6, 69; *P*= <0.0001) and number of inflorescences (F= 6.82, df= 6, 69; *P*= <0.0001) at White Bear Lake (Figure 3c-d) and decrease in purple loosestrife stem density (F= 3.25, df= 6, 73; *P*= <0.007), height ((F= 71.64, df= 6, 73; *P*= <0.0001), and number of inflorescences (F= 10.46, df= 6,73; *P*= <0.0001) at the Reno site (Figure 4b-d) corresponding and subsequent to increase in egg massess. The purple loosestrife rebounded, however, when *Galerucella* spp. declined at both sites (Figures 3a and 4a). Although there was some fluctuation of the number of plant species other than purple loosestrife at White Bear Lake (F= 6.68, df= 6,70; *P*= <0.0001), there was no difference between two and seven years after release (Figure 3e). There was no change in the number of plant species other than purple loosestrife at Reno (F= 2.23, df= 6,73; *P*= 0.05) over time (Figure 4e).

At Big Marine Lake, *Galerucella* spp. egg mass densities peaked four years after release (F= 13.38, df= 6,76; *P*= <0.0001) and declined sharply the following year (Figure 5). Following an initial increase in egg mass abundance three years after release, there was a marked reduction in purple loosestrife stems (F= 7.40, df= 6,76; *P*= <0.0001), height (F= 80.64, df= 6,76; *P*= <0.0001), and flowering (F= 26.76, df= 6,76; *P*= <0.0001)(Figure 5b-d). There was no change in the number of plant species other than purple loosestrife at Big Marine Lake (F= 1.93, df= 6,76; *P*= 0.087).

Dissections of *Galerucella* spp. collected from four of the five sites in 2004, suggest that two sites are dominated by *Galerucella calmariensis* (Circle Lake (90%) and Winona 94 %) and two sites are dominated by *G. pusilla* (Big Marine Lake (94%) and White Bear Lake (100%). No sample was obtained for the Reno location.

4. Discussion

At all five release locations, *Galerucella* spp. populations peaked between three and five years after successfully establishing. As a result, purple loosestrife densities, height and flowering were reduced across all sites, similar to the findings of Blossey and Skinner (2000), Landis et. al. (2003) and Lindgren (2003). Lindgren (2003) documented complete elimination of purple loosestrife stems, at one site, six years post release and a subsequent decline in *Galerucella calmariensis* abundance. In contrast, purple loosestrife remained present at all of our locations, albeit much reduced at three of the five locations. After the initial peak in *Galerucella* spp. densities, all sites saw a decline of *Galerucella* spp. abundance in response to the reduction in purple loosestrife abundance. After this initial peak, *Galerucella* spp. and loosestrife abundance followed two distinct patterns over time. The *Galerucella* spp. populations either rebounded with increasing loosestrife abundance or the Galerucella spp. population did not rebound.

At Winona and Circle Lake, *Galerucella* spp. populations rebounded (seven years post release) after egg mass densities neared zero. At Reno and White Bear Lake, *Galerucella* spp. populations have not rebounded since their initial declines six years post release and have remained low for two to three years. *Galerucella* spp. populations at all five sites suggest a density dependent relationship with purple loosestrife, but lack of a population rebound at Reno and White Bear Lake suggest that other factors may be influencing a population response. One such response may be stochastic effects that can occur with small insect populations that may cause small populations to go extinct locally. In particular, Allee affects and environmental variability play significant roles in insect establishment (Grevstad 1999a). Grevstad (1999a, 1999b) suggests that the combination of these two factors have an affect on establishment whereby establishment rate increases gradually with a concomitant increase in founder size.

Dominance by *Galerucella calmariensis* or *G. pusilla* may be reflected in beetle densities and control success at individual sites. At Circle Lake (nine years post release) and Winona (seven years post release) where insect abundance and purple loosestrife control was sustained, the dominant species was *Galerucella calmariensis*. The dominant insect species at Big Marine Lake (five years post-release) and White Bear Lake (eight years post-release) where control was not sustained, was *Galerucella pusilla*. It is suspected that the dominant species released at all sites was *G. calmariensis*. In particular, the beetles introduced to Big Marine Lake were collected from Circle Lake, where the dominant insect is *G. calmariensis*. We speculate that the initial increase in *Galerucella* spp. abundance at most sites may be dominated by *G. calmarienis*, but at some sites, such as Big Marine Lake and White Bear Lake, the smaller remaining populations are predominantly *G. pusilla*. Further research is required as to if and why this may have occured.

Plant species richness increased at sites where purple loosestrife control was realized long-term, indicating long-term control of purple loosestrife is a key element in sustaining a diversity of native plant species. Treberg and Husband (1999) and Farnsworth and Ellis (2001) found no association between number of native wetland species and purple loosestrife. However, after disturbance events, such a broadleaf herbicide application (Gabor and Murkin 1996) or establishment of biocontrol agents (Landis et al. 2003) number and or density of native species increased. Gabor and Murkin (1996) reported an increase in number of grass seedlings after broadleaf herbicide treatments on purple loosestrife compared with control treatments. Our finding were similar to Landis et al. (2003), who reported an increase in native species richness as purple loosestrife plant height and percent cover declined after establishment of *Galerucella* spp.

Although our results were similar to Blossey and Skinner (2000) and Landis et al. (2003) at three to five years post-release, *Galerucella* spp. abundance and control success may vary over the long-term. Meta population dynamics may influence the re-colonization of sites, where *Galerucella* spp. population declines have occurred. At the Winona and Circle Lake locations, there are multiple wetlands, infested with purple loosestrife, that surround our study site. Three years post-release, *Galerucella* spp. had spread from the study site to other purple loosestrife infested wetland up to 15 km away. We speculate that after a *Galerucella* spp. population declines and the purple loosestrife rebounded, immigration of *Galerucella* spp. from nearby wetlands may aid in their re-establishment. Further study, however, is needed to confirm this hypothesis.

In conclusion, *Galerucella* spp. can provide effective control of purple loosestrife. However, there may be limitations whereby some insect populations decline precipitously after reaching high densities and do not recover following declines or have not been observed to recover in the time frame of this study. Continued monitoring will be needed to determine of the Galerucella spp. populations will once again increase and control the purple loosestrife without reintroducing the beetles. Control of purple loosestrife can increase species richness when control is sustained. We have observed distinct benefits of *Galerucella* spp. as a biological control agent.

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Table 1. Site characteristics and *Galerucella* spp. release information.

Site	County	Latitude	Longitude	Site Type	Cowardin	Species	Release Date(s)	Number Released
Big Marine Lake	Washington	45.20536 N	92.86505 W	Wet Meadow, saturated soils; lakseshore	PEM/SS1B	GC,GP	1998	7000
						GC,GP	1999	21000
Circle Lake	Rice	44.42256 N	93.36604 W	Shallow Marsh, semi-permanently flooded	PEMF	GC,GP	1994	500
Reno	Houston	43.59517 N	91.29186 W	Shallow Marsh, semi-permanently flooded	PEMFh	GC,GP	1993	1000
						GC,GP	1995	4165
White Bear Lake	Ramsey	45.09389 N	93.00183 W	Shallow Marsh, seasonally flooded	PEMCd	GC,GP	1993	1000
						GC	1995	3306
						GP	1995	937
Winona	Winona	44.03871 N	91.64974 W	Shallow Marsh, seasonlly flooded	PEMC	GC,GP	1993	1000
						GC	1995	2184
						GP	1995	2091
						GC,GP	1996	6750

GC= *Galerucella calmariensis*, GP= *Galerucella pusilla;* Cowardin refers to wetland classification system (Cowardin 1979)

Table 2. Sampling design information for five *Galerucella* spp. release sites in Minnesota.

Site	Number of Transects	Total Number of Quadrats	Number of Years Sampled
Big Marine Lake	2	12	7
Circle Lake	6	30	9
Reno	2	12	7
White Bear Lake	2	11	7
Winona	2	14	7

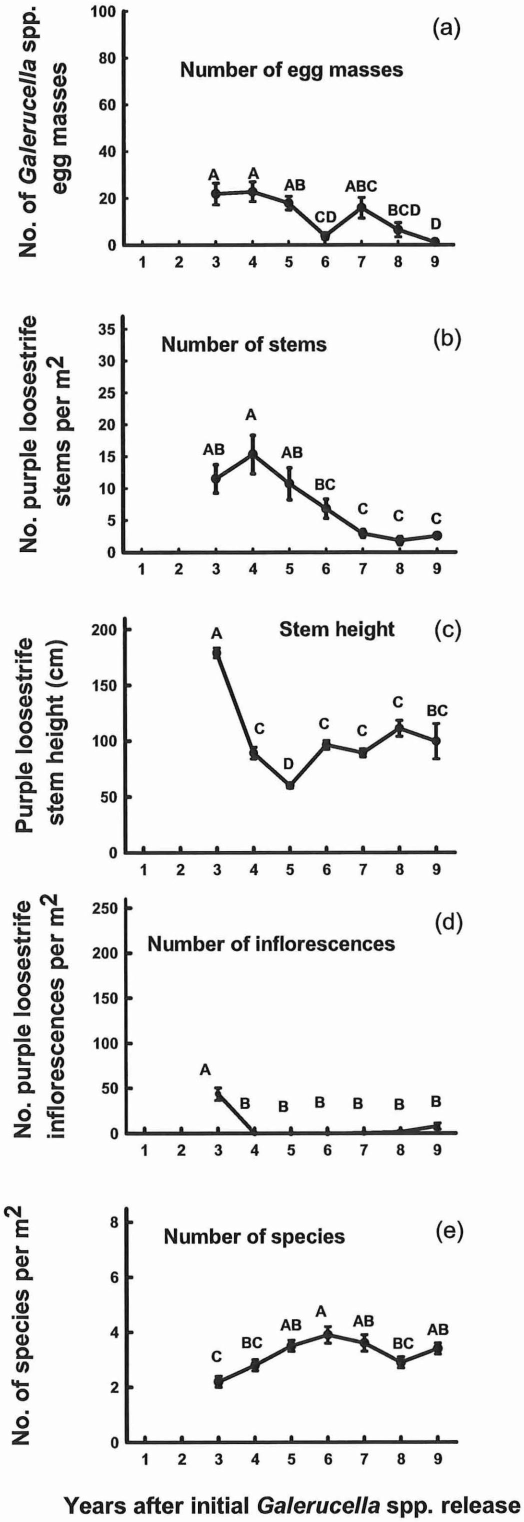

Fig. 1. Density of *Galerucella* spp. egg masses (a) and effect on purple loosestrife stem density m^2 (b), purple loosestrife stem height (c), purple loosestrife flowering (d), and number of plant species other than purple loosestrife (e), by year after release (Circle Lake, Minnesota). Within each figure, means with the same letter are not significantly different as determined by Ryan-Einot-Gabriel-Welsch Multiple Range Test (P<0.05). Error bars are ± standard error about the mean.

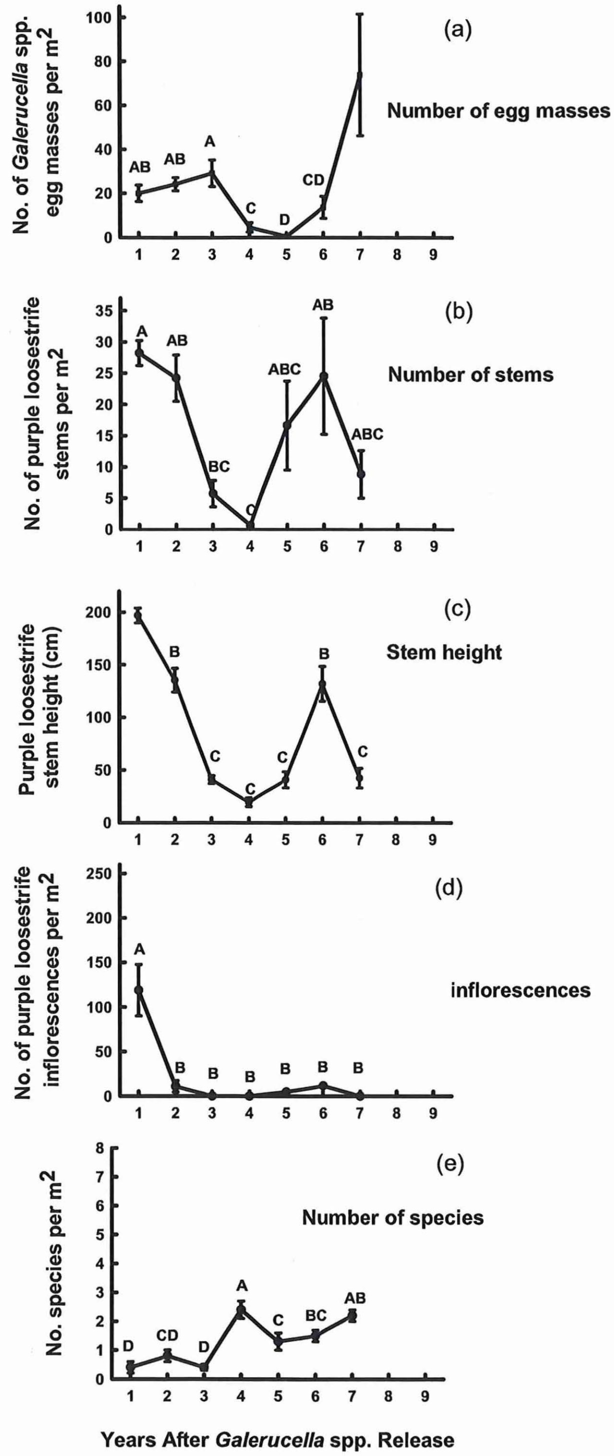

Fig. 2. Density of *Galerucella* spp. egg masses (a) and effect on purple loosestrife stem density m^2 (b), purple loosestrife stem height (c), purple loosestrife flowering (d), and number of plant species other than purple loosestrife (e), by year after release (Winona, Minnesota). Within each figure, means with the same letter are not significantly different as determined by Ryan-Einot-Gabriel-Welsch Multiple Range Test ($P<0.05$). Error bars are ± standard error about the mean.

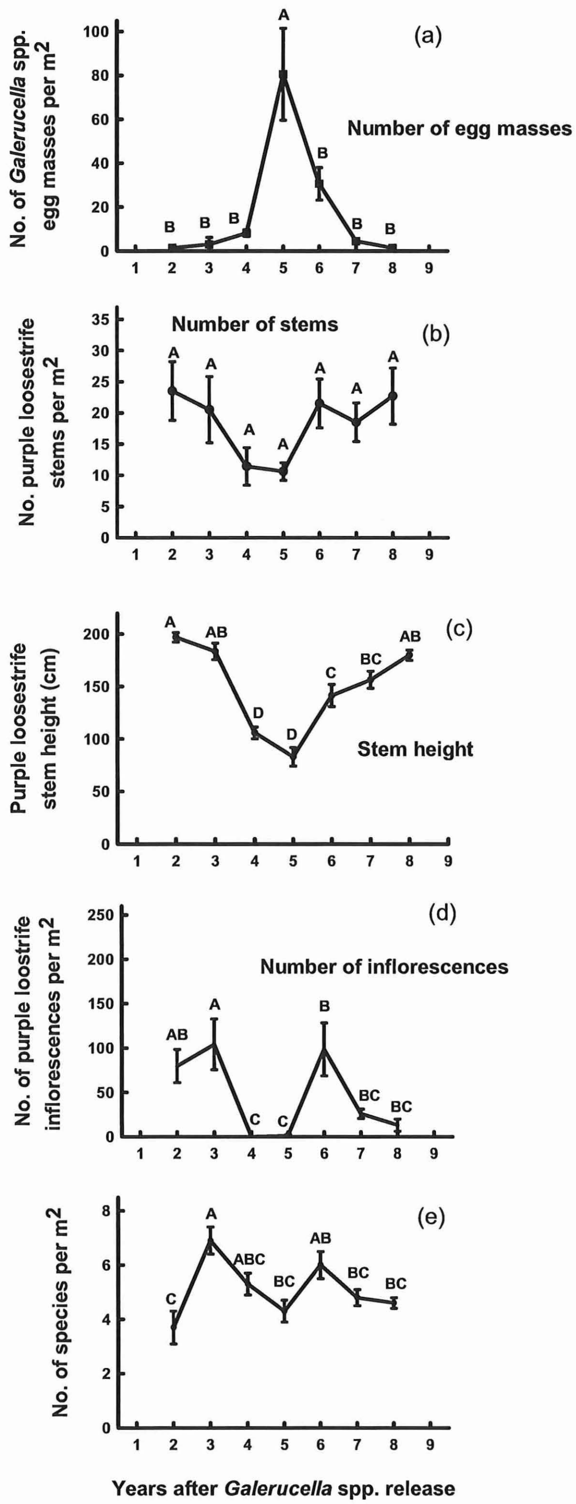

Fig. 3. Density of *Galerucella* spp. egg masses (a) and effect on purple loosestrife stem density m^2 (b), purple loosestrife stem height (c), purple loosestrife flowering (d), and number of plant species other than purple loosestrife (e), by year after release (White Bear Lake, Minnesota). Within each figure, means with the same letter are not significantly different as determined by Ryan-Einot-Gabriel-Welsch Multiple Range Test (P<0.05). Error bars are ± standard error about the mean.

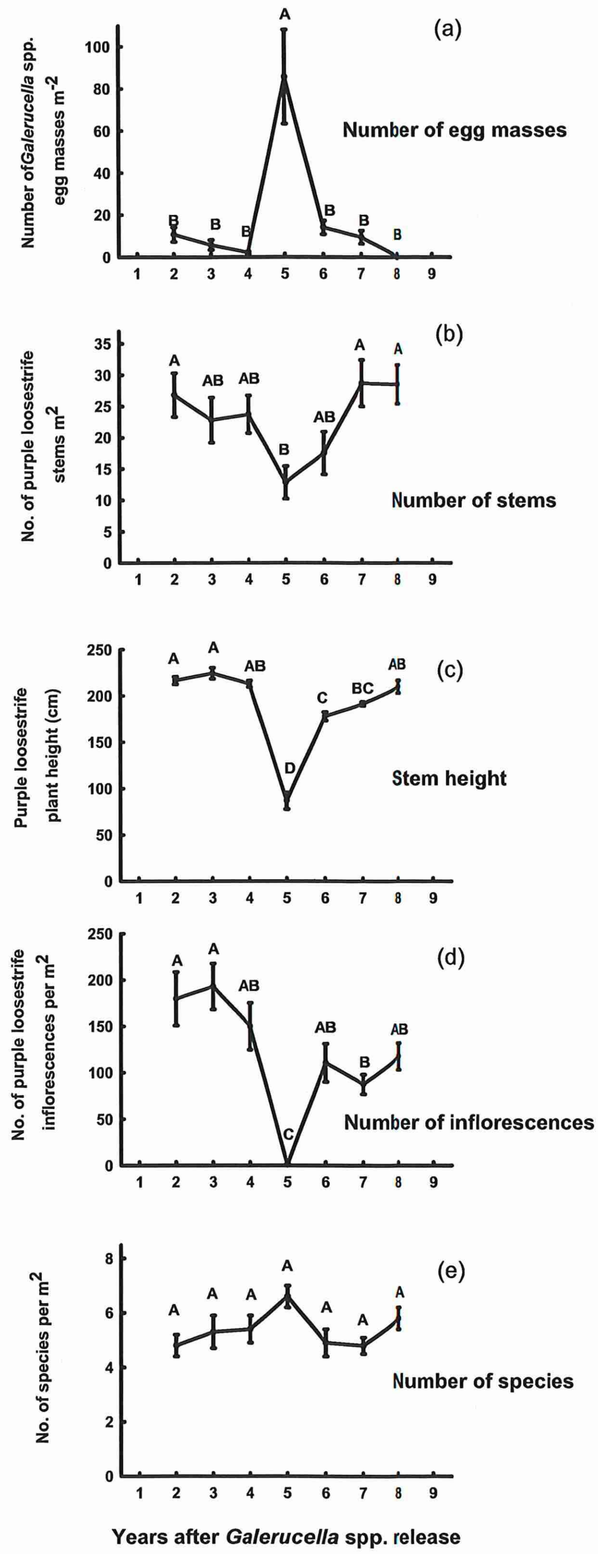

Fig. 4. Density of *Galerucella* spp. egg masses (a) and effect on purple loosestrife stem density m^2 (b), purple loosestrife stem height (c), purple loosestrife flowering (d), and number of plant species other than purple loosestrife (e), by year after release (Reno, Minnesota). Within each figure, means with the same letter are not significantly different as determined by Ryan-Einot-Gabriel-Welsch Multiple Range Test (P<0.05). Error bars are ± standard error about the mean.

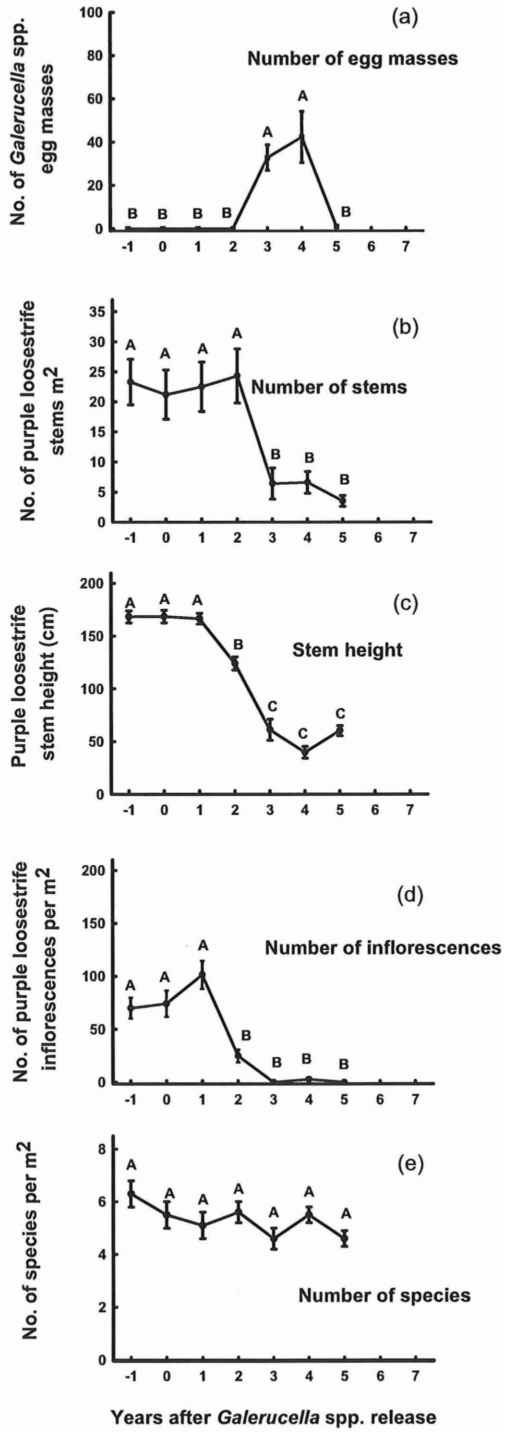

Fig. 5. Density of *Galerucella* spp. egg masses (a) and effect on purple loosestrife stem density m^2 (b), purple loosestrife stem height (c), purple loosestrife flowering (d), and number of plant species other than purple loosestrife (e), by year after release (Big Marine Lake, Minnesota). Within each figure, means with the same letter are not significantly different as determined by Ryan-Einot-Gabriel-Welsch Multiple Range Test ($P<0.05$). Error bars are ± standard error about the mean.

Final Report for Result 2, Activity 2
(2001 LCMR- Biological Control of Eurasian Watermilfoil and Purple Loosestrife-Continuation)

Growth and phenology of three Lythraceae species in relation to *Galerucella* spp.

BY

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Abstract

Previous studies have characterized the feeding, oviposition and larval development of the biological control insects, *Galerucella* spp., on non-target Lythraceae species, including two species native to Minnesota, winged loosestrife (*Lythrum alatum*) and swamp loosestrife (*Decodon verticillatus*). However, the impact of *Galerucella* spp. feeding on growth and seed production of the non-targets, winged loosestrife and swamp loosestrife, has not been reported. The objective of this study was to compare the phenology, growth and seed capsule production of winged loosestrife and swamp loosestrife, in relation to purple loosestrife (*Lythrum salicaria*), with and without the impact of *Galerucella* spp. Our study has documented minimal larval feeding on winged loosestrife and swamp loosestrife from the first generation of beetles in mid-June. Although *Galerucella* larvae were present on swamp and winged loosestrife, with one exception, none of the measured plant growth or reproductive parameters were reduced as a result of larval or adult *Galerucella* feeding. In the first year of the study, number of winged loosestrife seed capsules were reduced with *Galerucella* feeding compared to control plants. However, there were no *Galerucella* spp. present on winged loosestrife in the second year of the study. In Minnesota, flowering and seed development in swamp loosestrife occurs a month later than in purple loosestrife or winged loosestrife. Since *Galerucella* larval shoot tip feeding reduces the number of seed capsules formed on purple loosestrife, missing the main period of larval feeding in mid-June provides a degree of "phenological protection" for swamp loosestrife from *Galerucella* spp. feeding.

Introduction

Host specificity screening for potential weed biological control agents is designed to determine whether a potential biological control insect can complete its life cycle on a non-target plant in a no-choice testing system (McEvoy 1996). Prior to release of *Galerucella* spp. in North America for the biological control of purple loosestrife (*Lythrum salicaria*), host specificity tests were conducted in Europe and the United States (Kok et al. 1992, Blossey 1994). Results of the tests indicated that *Galerucella* spp. fed and oviposited on several species of *Lythrum*, including two species native to Minnesota, winged loosestrife (*Lythrum alatum*) (Blossey 1994, Kok et al. 1992) and swamp loosestrife (*Decodon verticillatus*) (Kok et al. 1992). However, the only non-target species that supported *Galerucella* larval development past the first instar was winged loosestrife (Blossey 1994, Kok et al. 1992).

Target and nontarget plants. Purple loosestrife is a perennial emergent wetland plant introduced to North America from Europe (Thompson et al. 1987). Purple loosestrife displaces valuable wetland plant species and is an extremely successful colonizer of disturbed wetland ecosystems (Thompson et al. 1987). This species is a herbaceous perennial and forms a woody crown from which new shoots emerge every year (Shamsi and Whitehead 1973). Seed dispersal, rather than vegetative reproduction is the major means of dissemination. It is estimated that each plant is capable of producing up to 2.7 million seeds per season (Thompson et al. 1987). The prolific seed production and subsequent seed rain leads to the creation of an extensive seed bank (Welling and Becker 1990). Once a seedbank is established, purple loosestrife more successfully colonizes disturbed and open sites than do native species (Welling and Becker 1993).

In North America, the most cosmopolitan native species of *Lythrum* is winged loosestrife, *Lythrum alatum*, which grows throughout the United States and Canada (Blackwell 1970; Cody 1978; Graham 1975). Winged loosestrife flowers are distylous (have two flower morphs) (Anderson et al. 1993b) and are also pollinated by large insects such as bees and butterflies (Levin 1970). Winged loosestrife grows to 1.0 m in height and may be distinguished from purple loosestrife by having one flower per leaf axil (Graham 1975). Winged loosestrife is often found growing in drier sites than purple loosestrife, although both species can inhabit the same wetland (Anderson and Ascher 1993a).

Swamp loosestrife or water willow (*Decodon verticillatus*) is also a North American native plant of the Lythraceae family and grows north to Canada and as far south as Louisiana Swamp loosestrife is a perennial species, tristylous and is self-compatable (Eckert and Barrett (1993). It is estimated that 30% of the progeny are the result of self-fertilization. Swamp loosestrife plants also reproduce vegetatively when stems contact moist soil and produce new shoots and adventitious roots (Eckert and Barrett 1993). This species grows in aquatic habitats similar to purple loosestrife. Shoots of swamp loosestrife exhibit an arching growth habit and flowers are arranged in dense clusters in leaf axils (Gleason 1952). Swamp loosestrife is classified as a species of special concern in Minnesota (Minnesota Dept. Nat. Resources 1996).

In addition to potential concerns of the effect of introduced biological control insects on related nontarget plants, concern exists regarding the possible deleterious effects of purple loosestrife itself on closely related native plants. Purple loosestrife flowers are tristylous (have three flower morphs) and are self incompatible (Anderson and Ascher 1994). Flowers are pollinated by large insects such as bees. In a wetland study, purple loosestrife pollen was preferred over pollen from winged loosestrife flowers by both bees and butterflies (Levin 1970) Pollen transfer from purple loosestrife to winged loosestrife reduced seed set in winged loosestrife and commonly occurred in the field (Brown and Mitchell 2001, Brown et al. 2002).

As a preferred pollen source, purple loosestrife may have a competitive advantage over winged loosestrife beyond the effect of vegetative competition (Brown and Mitchell 2001).

Biological control insects. *G. calmariensis* and *G. pusilla* are two leaf -defoliating beetles(Chrysomelidae) with similar life histories (Blossey et al. 1995a) and in 1992, were introduced into North America from Europe as biological control agents for purple loosestrife. The beetles cause severe leaf and shoot defoliation through larval and adult feeding (Hight and Drea 1991). In Minnesota, overwintered adult *Galerucella* spp. emerge in mid-May to early-June, depending upon spring temperatures and begin feeding on developing shoots of purple loosestrife plants. Adults oviposit on leaves and stems in egg masses of approximately 5 eggs. After hatching, the first larval instar moves to the shoot meristem where it feeds on developing leaves through the second larval instar (McAvoy et al. 1997). Third instar larvae move out of the meristem and feed freely on fully expanded leaves where the feeding damage is characterized as "window-pane" damage by feeding on the leaf mesophyll while leaving the waxy cuticular layer intact (Hight and Drea 1991). Larval development from egg hatch to pupation typically takes about 30 days to complete. In Minnesota, by mid- to late- June, third instar larvae will descend to the ground and pupate in leaf litter on the ground or in aerenchymous root tissue if plants are in standing water. Adult *Galerucella* spp. emerge in early- to mid- July. A portion of the adults will feed on remaining purple loosestrife plants or on seedlings and soon begin laying eggs that will produce a second generation. In Minnesota, there is generally one generation of beetles per year (Loos and Ragsdale 1998) although a partial second generation is common in the southern one-third of the state. Some F_1 adults will not reproduce but rather feed on available plants and then enter reproductive diapause by late- July (Loos and Ragsdale 1998).

In the continental United States, non-target feeding by biological control insects on native plants is almost exclusively restricted to closely related target plants within the same genus (Pemberton 2000). Even when biological control insects do not form self-sustaining populations on non-target plants, spill-over damage may occur when non-target plants are near high populations of biocontrol insects (Schooler et al. 2003). For example, slight feeding and ovipostion on winged and winged loosestrife was noted in a field study in Canada and represented a "short term spill-over effect" (Corrigan et al. 1998). *Galerucella* spp. also fed and oviposited on another species of Lythraceae, crepe myrtle (*Lagerstroemia indica*), in field studies but larvae were not able to complete development. From these results, it was concluded that the release of *Galerucella* spp. posed little risk to crepe myrtle in North America (Schooler et al. 2003).

After evaluation, the Technical Advisory Group (TAG) determined that the benefit of introducing *Galerucella* spp. for the control of purple loosestrife outweighed the risk of potential feeding on populations of winged or swamp loosestrife (Blossey 1994). In the United States, *Galerucella* spp. were first approved for release for the biological control of purple loosestrife in 1992.

In screening potential biological control agents, examining the "physiological host range" of non-target hosts may not be sufficient. An examination of the "ecological host range", which includes non-target plant phenology and life cycle is also critical (McEvoy 1996, Louda et al. 2003). Previous studies have characterized the feeding, oviposition and larval development of *Galerucella* spp. on non-target plants (Blossey 1994, Kok 1992, Corrigan et al. 1998, Schooler et al. 2003). However, the impact of *Galerucella* spp. feeding on growth and seed production of the non-targets, winged loosestrife and swamp loosestrife, has not been reported. *Galerucella* spp. larvae feed on developing meristems of purple loosestrife. This results in production of fewer seed capsules per inflorescence and fewer seeds per plant (Katovich et al. 2001) Phenological events, such as time of flowering and seed production, may provide an additional level of protection from non-target feeding. The objective of this study was to compare the phenology, growth and seed capsule production of two native species of Lythraceae, winged loosestrife and swamp loosestrife, in relation to purple loosestrife, with and without the impact of *Galerucella* spp.

Materials and Methods

Two studies were established in 2001, repeated in 2002 and were conducted at the St. Paul campus of the University of Minnesota. The first study was designed to determine the effect of *Galerucella* spp. on the growth and seed capsule production of purple loosestrife, winged loosestrife and swamp loosestrife. The second study was established to examine the phenology of *Galerucella* spp. in relation to the three Lythraceae species as well as phenological differences among the three plant species.

Effect of *Galerucella* spp. on growth and seed capsule production of winged, swamp and purple loosestrife. Winged, swamp and purple loosestrife seeds were planted in the greenhouse in a standard greenhouse mix (silt loam: sand: manure: peat, 1:1:1:1, v/v/v/v) in early spring of 2000 and 2001. In July 2000, plants of all three species were transplanted outside into individual mesocosms (plastic wading pools, 0.9m diameter and 0.2 m depth) and filled with a peat based potting mix. Eight plants of a single species were transplanted into individual mesocosms for a total of 2 mesocosms of each species and were placed in a random arrangement. The plants grew through the season and were overwintered to establish plants for treatment the following year. For overwintering, all plants were mulched with straw and wood chips to simulate the natural insulative cover in wetlands. In the spring of 2001, the following treatments were applied for each species; 1) a control where all plants in one mesocom were treated with the systemic insecticide, imidacloprid, to prevent *Galerucella* spp. feeding and 2) allowing feral *Galerucella* spp. to feed and oviposit on all plants in a free choice fashion. The experiment was repeated with a new set of plants that were planted outside in pools in July 2001. The second experiment was initiated in the spring of 2002. In May 2002, few feral *Galerucella* adults were present on *Lythrum* plants in the experiment. This may have been due to the removal of a reservoir of beetles from established purple loosestrife plants growing in an adjacent area that were removed for building construction. For this reason, approximately 700 *Galerucella* spp. adults were collected from a wetland and released on the periphery of the study area. The beetles were able to freely locate potential host plants and lay eggs. The amount of adult and larval feeding and number of egg masses were recorded for each plant species. At the end of the growing season, shoot dry weights were obtained and number of seed capsules were counted on one randomly selected inflorescence from each plant. In 2001, the number of seed capsules were counted on an inflorescence from four plants in each mesocosm. In 2002, an inflorescence from all eight plants in each mesocom were counted. The experiment was a randomized complete block design in a split plot arrangement with insecticide or no insecticide treatment as the main plot and plant species as subplot. Each treatment was replicated eight times with each replication being a single plant. Analysis of variance was performed on data and means were separated with a protected Least Significant Difference test. Data was tested, found to be homogenous and was not transformed.

Phenology of *Galerucella* spp., purple loosestrife, winged loosestrife and swamp loosestrife study. Wetland mesocosms were created so that all three species were grown under the same environmental conditions. Winged, swamp and purple loosestrife seeds were planted in the greenhouse and plants were transplanted into outdoor mesocosms the year preceding treatment as described in the previous experiment. Mesocosms were dug into the ground so that soil temperature was not altered by aboveground placement. Plants were placed in a random arrangement with all three species present in a single mesocosm, for a total of nine plants per pool, three of each species. In the fall, plants were mulched lightly and overwintered. Each mesocosm was replicated four times.

Beginning in April, 2001, date of shoot emergence was noted and number of crown buds were recorded for each plant on a weekly basis. Date of flower bud formation and flowering was also recorded for each plant. Date of adult *Galerucella* emergence was noted. In early June, the number of *Galerucella* egg masses was recorded for each plant as well as date of first larval feeding. Air temperatures were obtained from the University of Minnesota Climate Center. Growing degree days (GDD_{b10}) were estimated using a base air temperature of 10 C with no maximum temperature (Climatologic Working Group 2001). Although a base temperature for purple loosestrife is not described in the literature, base temperatures from other perennial species such as alfalfa and hemp dogbane were used as a point of reference (Sharratt et al. 1989; Ransom et al. 1998). The experiment was analyzed as a randomized complete block design. Data was subjected to Analysis of Variance and means separated with a Least Significant Difference test. Data was tested and found to be homogeneous and was not transformed. The experiment was repeated in 2002 with a new set of plants, which were planted outside in mesocosms in the July, 2001.

Results and Discussion

Effect of *Galerucella* spp. on growth and seed capsule production of winged, swamp and purple loosestrife study. Number of *Galerucella* spp. egg masses in early June of 2001 and 2002 was highest on purple loosestrife plants with an average egg mass counts of 120.4 and 123.0 per plant for 2001 and 2002 respectively (Table 1). Both winged and swamp loosestrife had significantly fewer egg mass counts. There were an average of 17.5 and 0 egg masses per plant in 2001 and 2002 respectively on winged loosestrife plants. Swamp loosestrife plants had an average of fewer than one egg mass present for both years.

All but one of the end of season parameters measured for purple loosestrife were reduced as a result of *Galerucella* spp. feeding compared with the insecticide treated control (Table 2). Aboveground shoot biomass, plant height and number of seed capsules were reduced as a consequence of *Galerucella* feeding. The number of shoots at the end of the season was higher in plants with *Galerucella* feeding. This was most likely due to the release of crown buds as a result of diminished main shoot apical dominance caused by shoot defoliation.

There were no differences in dry weights of winged loosestrife shoots, plant height or number of shoots at the end of the season with or without *Galeurcella* feeding. In 2001, the number of seed capsules per inflorescence were reduced on plants with *Galerucella* feeding as compared to the insecticide control. However, in 2002, there were no differences in the number of seed capsules between treatments. In 2001, egg masses were present on 88% of winged loosestrife plants. In 2002, egg masses were not present on any winged loosestrife plants. The reason why egg masses were present on winged loosestrife plants in 2001 and not in 2002 is not known. However, in 2002, a different source of beetles were used in the study.

In both years of the experiment, swamp loosestrife plants had an average of fewer than one *Galerucella* egg mass per plant and little, if any, larval feeding damage. End of season shoot dry weight, number of shoots and seed capsules did not differ between the two treatments. Plant height was the only parameter which differed between treatments. Plants exposed to *Galerucella* spp. were shorter than the insecticide control plants. However, since there was little, if any non-target feeding visible on these plants, feeding by *Galerucella* does not appear to be the cause of the shorter plants.

Phenology of *Galerucella* spp. in relation to that of purple loosestrife, winged loosestrife and swamp loosestrife. In the spring of 2001 and 2002, the average date of purple loosestrife shoot emergence from crown buds occurred on April 17 when the average number of accumulated GDD_{b10} was 37 (Table 3). Shoots of winged loosestrife and swamp loosestrife emerged later than purple loosestrife shoots and were first observed on May 10 and May 16 respectively. At this time, accumulated GDD_{b10} were 178 and 211 for winged loosestrife and swamp loosestrife respectively. It is not known whether the spring emergence of shoots from crown buds of winged or swamp loosestrife is influenced by temperature or photoperiod as in other perennial species (Becker and Fawcett 1998). Number of shoots emerging from crown buds was notable higher for purple loosestrife plants than the other species (Figure 1). Additionally, the rate of shoot emergence from crown buds was notably faster for purple loosestrife, indicating the early resource capture of light and the potential for site domination of purple loosestrife.

The initial date of purple loosestrife flowering varies among regions and among populations within regions (Olsson and Agren 2002). In our study, flower buds were first observed on purple loosestrife on June 6 in 2001 (Table 3). In 2002, all purple loosestrife shoots were defoliated by *Galerucella* spp. so flowering was delayed. However, past studies show similar purple loosestrife flowering dates in Minnesota (Katovich et al. 1998). Purple loosestrife plants requires a critical day-length of 13 h for flower initiation and stem elongation to occur (Shamsi and Whitehead 1973). In St. Paul, MN (latitude 44^{o} $99^{'}$ N, longitude 93^{o} 21' W, 280 m above sea level) a 13 h daylength was reached on April 5, 2001 and April 6, 2002. This means that a critical daylength of 13 h was reached prior to emergence of crown buds from the soil in the spring. Flowering at the latitude of St. Paul, MN is probably not triggered by a change in daylength as the critical daylength was achieved approximately two months prior to crown bud emergence in the spring.

As seen for shoot emergence from crown buds, initiation of flower buds occurred later in winged loosestrife and swamp loosestrife compared with purple loosestrife. (Figure 2). Flower buds were first observed in winged loosestrife on June 23 (813 GDD_{b10}) and on July 18 for swamp loosestrife (1481 GDD_{b10}) compared to 510 GDD_{b10} for purple loosestrife. Date of flowering of the three species was defined as the time when the first flowers had opened. Purple loosestrife has an indeterminate inflorescence and flowering occurs until the end of the growing season. The first completely opened flowers were first noted for purple loosestrife and winged on June 28 (995 GDD_{b10}) and June 30 (1035 GDD_{b10}) respectively. However, flowers did not open in swamp loosestrife until August 2 (1876 GDD_{b10}). The date of the first fully opened flowers in swamp loosestrife occurred a full month later than the other two species.

Galerucella spp. adult feeding was first observed in all three species in late May (May 23 for purple loosestrife and winged loosestrife and May 27 for swamp loosestrife). Egg masses were present about a week later (Figure 2). First instar larval feeding was first observed in the middle of June on all three plant species and F_1 adults had emerged by July 11. Similar phenologies of *Galerucella* spp. life stages were recorded by Lindgren in Manitoba (2003).

As expected, larval feeding by *Galerucella* spp. resulted in fewer seed capsules on purple loosestrife inflorescences because of shoot tip and flower bud damage (Katovich et al. 2001). Under Minnesota climatic conditions, there is usually one generation of beetles per year (Loos and Ragsdale 1998). Swamp loosestrife plants flowered and set seed in August (Figure 2). The delayed phenological development of swamp loosestrife plants, compared with the other two species, resulted in avoidance of the first and second larval instar shoot tip feeding damage caused by the first generation of *Galerucella* spp. beetles (Figure 2). As a result, the shoot meristems and developing flower buds of swamp loosestrife were not damaged as they missed the larval damage that could have resulted in a reduction in number of seed capsules. In regions south of St. Paul, MN, *Galerucella* may produce more than one generation of beetles per year. Flowering in swamp loosestrife may coincide with later generations of *Galerucella* spp. larval feeding.

Differences in growth among Lythraceae species. Absent *Galerucella* spp., plant height in mid- July was greatest for purple loosestrife (Table 4). Total plant dry weight at the end of the season was approximately four times greater for purple loosestrife than the other species. Also, number of seed capsules averaged 239 capsules per inflorescence for purple loosestrife verses 75 and 23 capsules per inflorescence for winged loosestrife and swamp loosestrife, respectively. Estimates of purple loosestrife seed production per plant range from 600,000 (Cutright 1986) to 2,000,000 (Thompson et al 1987). Brown et al. (2002) determined that each winged loosestrife seed capsule produced 63 seed. From this, we estimate that each winged loosestrife plant produced approximately 147,000 seeds per plant in our study. Dorken and Eckert (2001) found an average of 1139 seeds per plant in swamp loosestrife. Our results show that if winged loosestrife or swamp loosestrife were growing the same wetland with purple loosestrife, purple loosestrife would produce considerably more seed per plant per year than the native species. This would eventually overwhelm all other plant competitors through seedling recruitment, as shown by Welling and Becker (1990) who estimated that 400,000 purple loosestrife seed m^{-2} were present in the upper 5 cm of a Minnesota wetland.

Initial host specificity studies with the native non-target species, winged loosestrife and swamp loosestrife demonstrated that *Galerucella* spp. would feed, oviposit, and in the case of winged loosestrife, larvae would develop to the first instar stage (Blossey 1994, Kok et al. 1992). Our study has documented minimal larval feeding on winged loosestrife and swamp loosestrife from the first generation of beetles in mid-June. Although *Galerucella* larvae were present on swamp loosestrife, none of the measured plant growth or reproductive parameters were reduced as a result of larval or adult *Galerucella* feeding. In addition, in Minnesota, flowering and seed development in swamp loosestrife occurs a month later than in purple loosestrife or winged loosestrife. Since *Galerucella* larval shoot tip feeding reduces the number of seed capsules formed on purple loosestrife (Katovich et al. 2001), missing the main period of larval feeding in mid-June provides a degree of "phenological protection" for swamp loosestrife from *Galerucella* spp. feeding.

In the first year of our study, the number of seed capsules were reduced by 31% on winged loosestrife plants compared with an insecticide treated control. No other plant growth parameters were reduced. However, in the second year of our study, no *Galeucella* beetles or egg masses were present on winged loosestrife plants the entire season and number of seed capsules were not reduced. By contrast, with *Galerucella* spp. feeding, there was a 64% reduction of the number of seed capsules produced by purple loosestrife. In a wetland where

70% of purple loosestrife leaves where defoliated by *Galerucella*, few to no purple loosestrife flower buds and seeds were produced (Katovich et al. 2001). Thus, the potential exists for a great reduction of purple loosestrife seeds by *Galerucella* spp. feeding with little or no reduction in the native, nontarget Lythraceae.

Purple loosestrife is a highly competitive plant compared with winged and swamp loosestrife. This is evident from phenological characteristics, such as earlier spring emergence, and greater number of shoots emerging from crown buds in the spring. Other growth traits, such as greater plant height and above ground biomass were higher in purple loosestrife, compared with swamp or winged loosestrife, and have been correlated with a greater competitive ability in purple loosestrife (Gaudet and Keddy, 1988). Purple loosestrife also has a greater potential for seed production compared with the other two plant species and Weihe and Neely (1977) found that the number of reproductive structures was an indicator of competitive success in purple loosestrife. Due to the highly competitive growth characteristics of purple loosestrife, it may be argued that there is a greater benefit from release of *Galerucella* in wetlands compared with the minimal non-target feeding and ovipostion effects on winged loosestrife and swamp loosestrife.

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Table 1. Number of *Galerucella* spp. egg masses present on three species of Lythraceae, June 2001 and 2002. St. Paul, MN

Species	Number of *Galerucella* spp. egg mass	
	2001	**2002**
Purple loosestrife	120.4	123.0
Winged loosestrife	17.5	0
Swamp loosestrife	0.8	0.3
(LSD 0.05)	26.7	42.2

Table 2. Shoot dry weights, plant heights, number of shoots and seed capsules of three species of Lythraceae with and without *Galerucella* spp. feeding, St. Paul, MN, 2001 and 2002.

Feeding status	**End of season shoot dry weight**	**End of season plant height**	**Seed capsules**		**Shoots**
Purple loosestrife	(g)	(cm)	(no.)		(no.)
no *Galerucella* feeding	86.9	150.3	258.8		7
with *Galerucella* feeding	56.3	104.6	92.9		11.1
LSD (0.05)	28	12.8	56.3		3.7
Swamp loosestrife					
no *Galerucella* feeding	21.8	114.6	21.1		5.5
with *Galerucella* feeding	30.6	84.9	28.6		5.5
LSD (0.05)	NS	28	NS		NS
Winged loosestrife			2001	2002	
no *Galerucella* feeding	12.2	62.8	105	48.5	31.1
with *Galerucella* feeding	10.2	54.9	72.3	55	28.3
LSD (0.05)	NS	NS	24.2	NS	NS

<u>Table 3</u>. Crown bud emergence time, initiation of flower buds and date of first flowering, 2001 and 2002.

	Purple loosestrife			Winged loosestrife			Swamp loosestrife		
	Date	Julian Date	GDD_{b10}	Date	Julian Date	GDD_{b10}	Date	Julian Date	GDD_{b10}
emergence	4-17	107	37	5-10	130	178	5-16	136	211
flower bud	6-6[1]	157	510	6-23	174	813	7-18	199	1481
flowering	6-28[1]	179	995	6-30	182	1035	8-2	214	1876

[1]Based on 2001 results only

<u>Table 4</u>. Growth differences among species of Lythraceae

Species	Plant Height	Numbers of stems	Number of seed capsules per inflorescence	End of season dry weight
	(cm)			(g)
Purple loosestrife	105	7	239	80
Winged loosestrife	70	31	75	15
Swamp loosestrife	61	6	23	21
LSD (0.05)	14	9	26	8

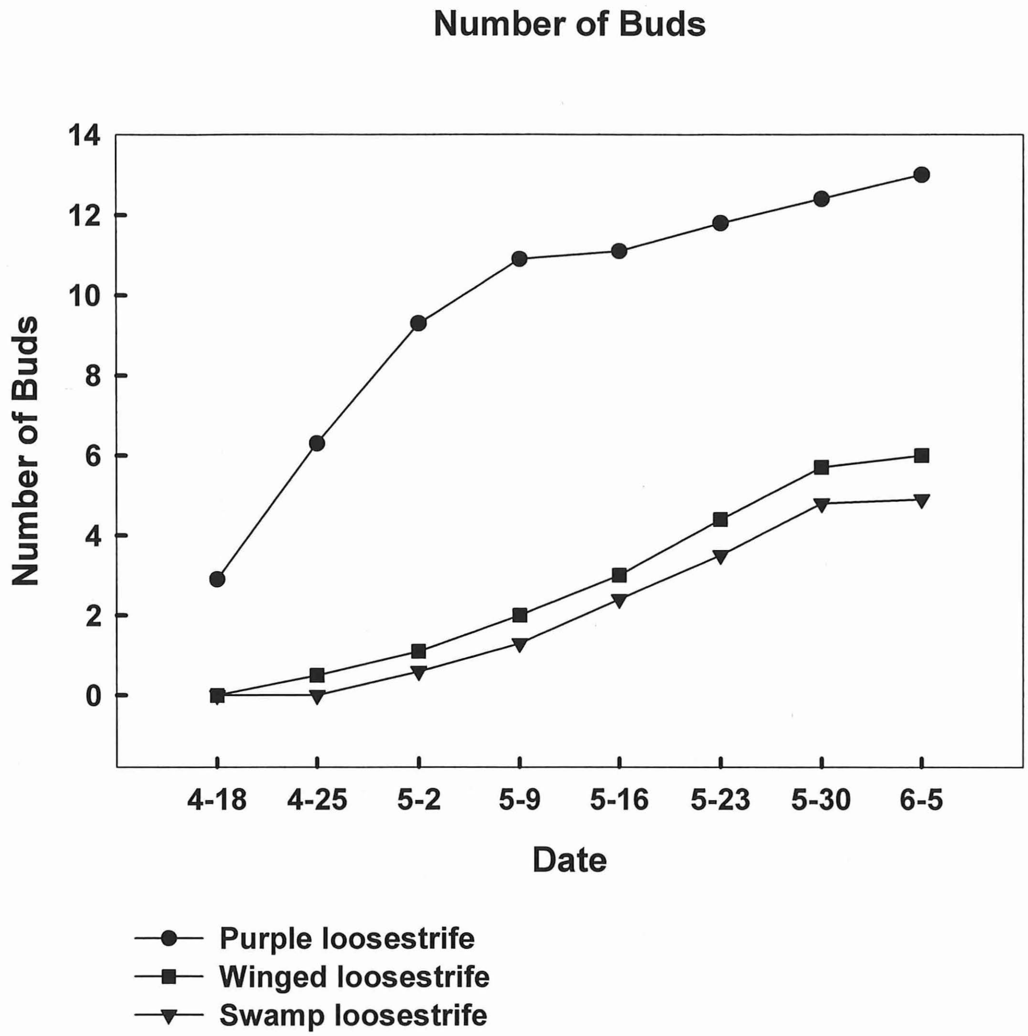

Figure1. Number of shoots emerging from crown buds for purple, winged and swamp loosestrife.

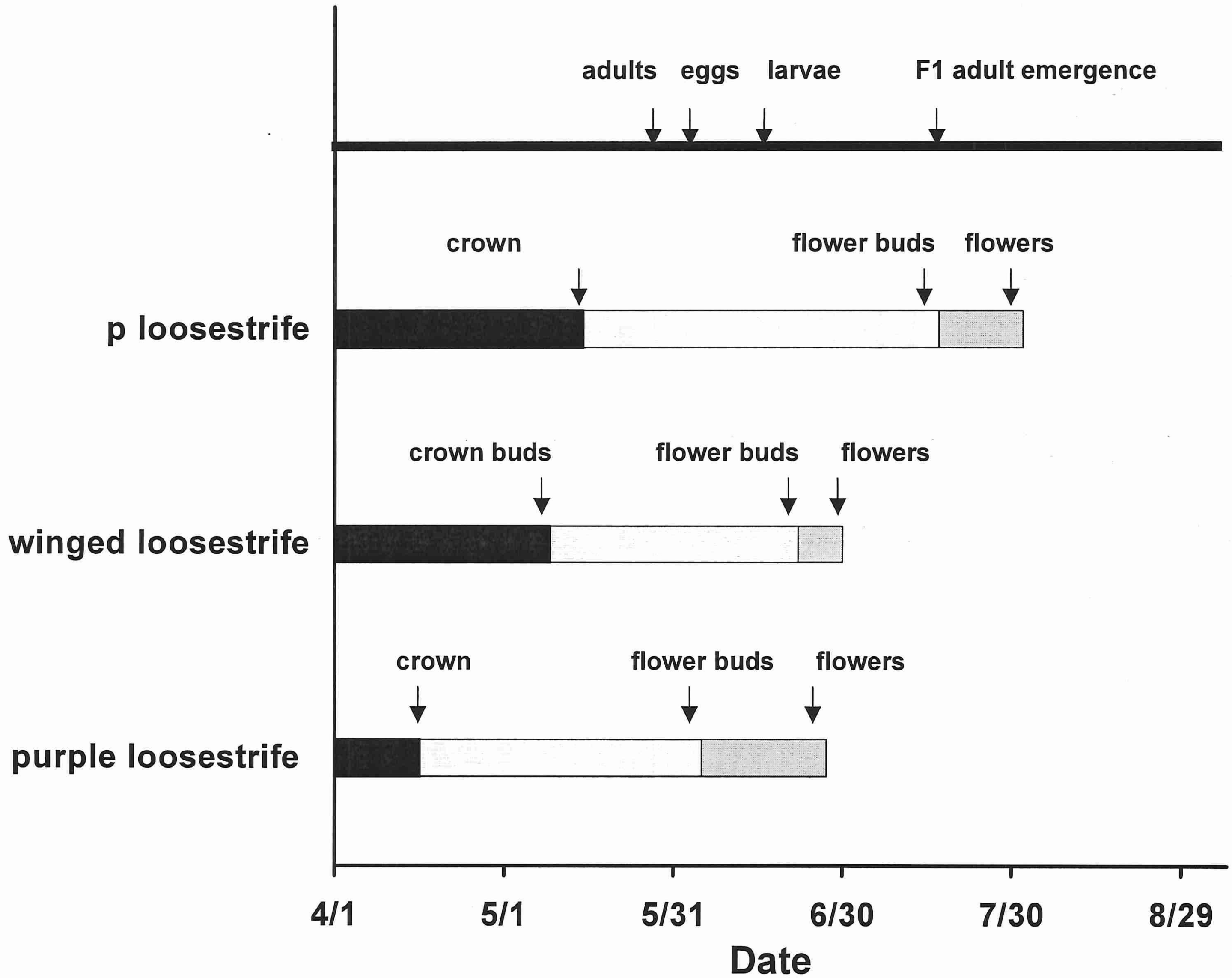

Figure 2. Phenology of purple, winged and swamp loosestrife, and the leaf-feeding beetle, Galerucella spp.

Final Report for Result 3
(2001 LCMR- Biological Control of Eurasian Watermilfoil and Purple Loosestrife-Continuation)

Landscape-Scale and Within-Wetland Movement of *Galerucella* spp. Introduced for Management of Purple Loosestrife (*Lythrum salicaria* L.)

BY

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ABSTRACT

In 1992, leaf beetles *Galerucella calmariensis* and *G. pusilla* were introduced from Europe as biological control agents against purple loosestrife, *Lythrum salicaria* L. in the United States. The ability of *Galerucella* spp. to control or reduce purple loosestrife infestations has been well documented. However, there is limited knowledge regarding the ability of this insect to disperse, and a technique often used to study insect spatial distributions is geostatistics. The objectives of this study were to 1) characterize the spatial distribution of *Galerucella* spp. within a wetland, and 2) evaluate the ability of *Galerucella* spp. to disperse to noncontiguous loosestrife infested wetlands on a landscape-scale. *Galerucella* spp. disperse and colonize a wetland habitat shortly after the initial release. In our experiment, apparent reductions in purple loosestrife infestations were often related to high egg mass densities of *Galerucella* spp. egg masses and beetle damage observed in the spring. This trend was present in all four wetlands studied. These beetles appear to be well adapted to changing environments and are capable of dispersing and colonizing large purple loosestrife infestations. On average, beetles dispersed 5 km from established release sites to non-release sites within 3 years. To maximize redistribution efforts, we advise resource managers to select wetlands that are greater than 5 km from known release sites. *Galerucella* spp. is capable of colonizing new purple loosestrife infestations, thus reducing redistribution efforts from resource managers.

Keywords: *Galerucella* spp., purple loosestrife, biological control, geostatistics

INTRODUCTION

In 1992, leaf beetles *Galerucella calmariensis* and *G. pusilla* were introduced from Europe as biological control agents against purple loosestrife, *Lythrum salicaria* L. These two species cannot be reliably identified in the field and dissection of male genetalia is necessary for species determination. Here we report field observations and thus are reporting distribution of *Galerucella* spp. These beetles inhabit similar niches and have similar phenologies (Blossey 1995). The ability of *Galerucella* spp. to control or reduce purple loosestrife infestations has been well documented. However, there is a limited knowledge regarding the ability of this insect to disperse (Grevstad and Herzig 1997). Grevstad and Herzig (1997) showed that beetles could disperse up to 1 km within a short time period along a contiguous stand of loosestrife in roadside ditches. However, long-range dispersal over areas that do not contain purple loosestrife and spatial distributions within larger infested wetlands is not known. Successful biological control programs over a region are dependent upon the biocontrol agent to disperse to noncontiguous host plant patches. Documentation of the movement of biocontrol agents on a landscape scale has not been done with *Galerucella* spp. Here we describe movement of insects observed throughout a wetland and among wetlands that exist as isolated patches of host plants.

A statistical technique used to study spatial distribution of various organisms is geostatistics. The use of geostatistics to answer entomological questions regarding dispersal of insects has increased within the past ten years, and as a result, geostatistical techniques have been used to describe within-field spatial structures of many insect systems (Williams et al. 1992, Midgarden et al. 1993, Darnell et al. 1999, Schotzko and Quisenberry 1999, Barrigossi et al. 2001, Blom et al. 2002, Dávalos and Blossey 2004). In general, "the degree of association (correlation) between samples is based on the direction and distance between them" (Schotzko and Quisenberry 1999). Thus, geostatistics provide a new approach to describe variability between spatially separated samples (Rossi et al. 1992). The closer the points are geographically, the greater the chance of spatial relatedness (Liebold et al. 1993). In geostatistics, a semivariogram is used to plot distances "between sample pairs against a semivariance statistic (variation between two points) for all possible sample pairs at each distance" (Ellsbury et al. 1998). Kriging is an interpolative technique that describes these spatial relationships across the landscape (Liebold et al. 1993).

The objectives of this study were to 1) characterize the spatial distribution of *Galerucella* spp. within a wetland and 2) evaluate the ability of *Galerucella* spp. to disperse to noncontiguous loosestrife infested wetlands on a landscape-scale.

MATERIALS AND METHODS

Within-wetland beetle movement. The spatial distribution of *Galerucella* spp. was characterized in four wetlands heavily infested with purple loosestrife, which were ideal for our long-term, small-scale dispersal study. The first two sites in the study, referred to as Frontenac Lake (UTM X:552768, Y:4928465) and Wacouta Pond (UTM X:546798, Y:4930447), were located in Goodhue County, MN. A third site, referred to as Sherburne Pool (UTM X:446755, Y:5034811), was located in the Sherburne National Wildlife Refuge in Sherburne County, MN. The final site used in the study was located in Hennepin County, MN and is referred to as Stonebridge Road (UTM X:463979, Y:4977743).

Spring sampling. Varying densities of *Galerucella* spp. beetles (4000 to 37,000) were released into Frontenac Lake in 1998, Wacouta Pond in 1999, Sherburne Pool in 1999, and Stonebridge Road in 2001. The initial release points for each wetland are noted in Fig. 1.

Within each wetland, waypoints were staked with polyvinyl chloride (PVC) pipe in a grid pattern (i.e., ≈ 25-m spacing between points in all cardinal directions). Global Positioning System (GPS) coordinates were recorded for each waypoint using a Garmin® 12 GPS Map. In the spring of 2001, 2002, and 2003 the number of purple loosestrife crowns in a 2-m radius and the number of these crowns showing beetle damage were recorded. The tallest stem from each of the closest 10 crowns at each waypoint was collected and the total number of egg masses/stem was recorded.

Fall sampling. In the fall of 2002 and 2003, plant biometrics (i.e., measurable plant characteristics) were assessed to describe purple loosestrife damage within each wetland. Biometrics included: height and number of inflorescences/stem for the five tallest stems/m^2, total number of stems/m^2, total number of inflorescences/m^2, and percent cover.

Geostatistics. Point maps were created in the GIS ArcMap 8.2 (Environmental Systems Research Institute 1999) to predict distribution of all variable measured in 2001, 2002, and 2003. Spatial autocorrelation was determined using the variogram analysis in GS+ (Gamma Delta Software, Plainwell MI). Distributions of all datasets were tested for normality using the Royston (1992) modification to the Shapiro–Wilk W-test (Shapiro and Wilk 1965) (PROC UNIVARIATE, SAS Institute 2001). Prediction maps for beetle egg mass density and plant biometrics were interpolated using ordinary kriging in ArcMap 8.2. Maps were visually compared to investigate the impact of *Galerucella* spp. on purple loosestrife infested wetlands.

Landscape-scale beetle movement. In 2001 and 2002, four geographic regions in Minnesota that contained numerous, loosestrife infested wetlands with at least one release site where purple loosestrife was being reduced by beetle feeding. The areas used in our study were located in the following county clusters: Swift/Pope Counties, Wright/Carver/Hennepin Counties, Anoka/Ramsey/Chisago/ Washington Counties, and Goodhue/Wabasha Counties (Fig. 2). A database containing all known purple loosestrife infestations maintained by the Minnesota Department of Natural Resources was used to locate regions of the state that met the above criteria. We used the same database to locate wetlands that had beetles released. We visted these infested sites, to determine the level of plant damage caused by all life stages of the *Galerucella* spp. beetles. At each site visited, randomly selected purple loosestrife plants (100 maximum per site) were assessed for insect presence (i.e. defoliation, eggs, larvae, adults). The *Galerucella* spp. life stages present and the type of damage observed (i.e., shot-hole and tip feeding, reduced flowering) were recorded and GPS coordinates were recorded at each site (Fig. 3). Purple loosestrife density and plant numbers were estimated and recorded for each site. Once overall damage was assessed, a letter grade ranging from A-F (A = highest percent damage with an abundance of insects and extensive plant damage. Insects from a site with a grade of A can be repeatedly collected and redistributed, B = insects were commonly found and insects could be collected and redistributed, plant damage is observable, but not a dramatic reduction in plant stand, C = insects can be found, but plant damage is modest and beetle density too low to collect and redistribute, D = occasional insects can be observed, but virtually no or only limited plant damaged can be found, and F = no plant damage and absence of beetles) was given to each site. The letter grades are used as a guide to when insect density and damage is sufficient to begin removing insects from the site for redistribution. The overall visual appearance of the plants in the spring and again in the summer after flowering is a key indicator of the success of biocontrol agents used by practitioners who manage.

Sites that received a grade of C or higher (on an A-F scale) in 2001 were not surveyed in 2002. However, sites receiving a grade of D or F in 2001 were re-sampled in 2002. Each year new purple loosestrife infestations not previously graded were also added as they were discovered. Using GIS, when an apparent early colonization (beetles present but damage low) was discovered on a non-release site, the distance to the closest release site was determined. By evaluating the insect population from the closest source we estimated the number of years it took for beetles to colonize these non-release sites and used ArcMap 8.2 to spatially analyze these data. For each region, we calculated mean dispersal distance (km), maximum dispersal distance (km), mean number of years to detect beetle presence, and the proportion of all non-release sites visited with beetles present.

RESULTS

Within-wetland beetle movement. Significant spatial correlations were present in nearly all the datasets and semivariograms for all data are presented in Table 1. However, when data are not spatially correlated, interpreting kriged surfaces is not possible. Instead, the mean value between all waypoints can be used to describe unknown locations within a site. At Wacouta Pond in the spring of 2001, high egg mass densities were localized around the initial release point (Fig. 4A). However, low to moderate damage (i.e., percent crown damage) was evident in over 80% of the wetland (Fig. 4D). By the spring of 2002, egg mass densities were observed across the entire wetland; the greatest concentration of egg mass densities (i.e., 20-30 egg masses/stem) were found near or at the release point (Fig. 4B). Percent crown damage increased across the entire wetland compared to the previous year with 50-75% of the purple loosestrife crowns in a 2 m radius of the waypoint showing damage (Fig. 4E). When comparing egg mass densities to plant biometrics that were measured in the fall at Wacouta Pond, areas of heavy oviposition were correlated to a reduction in percent purple loosestrife coverage (Fig. 5A), average stem height (Fig. 5C), mean number of inflorescences (Fig. 5E), and total number of inflorescences/m^2 (Fig. 5I). There were no inflorescences found in over half of Wacouta Pond in 2002.

In 2003, we started to see a shift in the location of high egg mass densities within Wacouta Pond (Fig. 4C). The largest amount of *Galerucella* spp. egg masses was found ≈ 150 m south of the initial release point. Insect presence (i.e., shot-hole and tip feeding) was observed across the entire wetland with 75-100% of the crowns showing beetle damage (Fig. 4F). Egg mass densities reported in the spring of 2003 were visually similar to changes in plant biometrics measured in the fall. Since egg mass densities observed in the spring were lower than previously observed near the initial release points we expected to see less plant damage. Indeed we observed a rebound in the number of flowering stems near the initial release point (Fig. 5F,H) compared to the previous year, confirming that egg mass density in the spring appears to be a good predictor of overall plant damage seen late in the summer and into the fall. However, it should be noted that the overall height and the number of inflorescences was reduced across the entire wetland compared to those biometrics measured in 2002 (Fig. 5C-D).

At Sherburne Pool we observed similar trends. In 2001, there were low egg mass densities across the wetland plus we were unable to find *Galerucella* spp. eggs in the northeast half of the wetland and the southeast half of the wetland ranged from 1-5 egg masses/stem (Fig. 6A). Though no egg masses were found in the northeast half, evidence of feeding was observed across the entire wetland with 1-25% of the purple loosestrife crowns having damage (Fig. 6D). In 2002, egg mass densities slowly continue to spread across the wetland with two areas identified in the grid sampling as having substantially more egg masses (10-15 egg masses/stem) which were close to the initial release point (Fig. 6B). Adult activity was also greater and there was an overall increase in percent crown damage (Fig. 6D). However, despite the increase of egg masses in isolated areas, we do not see the same trend in plant biometrics as we did at Wacouta Pond in 2002. In contrast, percent cover, stem height, and number of inflorescences were apparently unaffected by the increase in egg mass densities (Fig. 7A,C,E,G,I).

By 2003, egg mass densities at the Sherburne Pool generally increased across the entire wetland averaging 5-10 egg masses/stem compared to densities that were 50% lower in 2002. In general, crown damage remained unchanged and the most damage was observed near the west edge of the wetland (Fig. 6F). When we relate this to the fall plant biometrics, we see similar trends that were observed at Wacouta Pond in 2003. High egg mass densities corresponded to a reduction in plant height (Fig. 7D) and a large reduction in the number of flowering plants (Fig. 7F,G). The total number of inflorescences was greatly reduced in 2003 compared to 2002; average number of inflorescences/m^2 in 2002 was > 25/m^2 and in 2003 there was only a small patch of purple loosestrife that had an average of 1-10 inflorescences/m^2 (Fig. 7G, H).

At the Stonebridge Road site, eggs were found throughout the wetland both years (Fig. 8A-B). In general, larger egg mass densities were located in the middle of the wetland and densities increased from 1-5 masses/stem in 2002 (Fig. 8A) to 20-25 masses/stem (Fig. 8B) in 2003. A similar trend was observed when comparing percent crown damage (Fig. 8C-D). As seen in the previous two sites, egg mass densities correlate with fall plant biometrics where higher egg mass densities correspond to reduction in percent cover, stem height, number of inflorescences, and number of stems both years (Fig. 9). We also observed this trend between years within a given biometric (i.e., greater egg mass densities resulted in a greater reduction in percent cover, plant height, and number of inflorescences).

At Frontenac Lake in 2001, egg mass densities and percent crown damage were highest near the release point and lowest at the opposite end of the wetland, some 300 meters distant (Fig. 10A,D). However, in 2002 an area of increased egg mass density was observed away from the release point (Fig. 10B). In the north half of the site percent crown damage increased from 0-25% damage in 2001 to 75-100% damage in 2002. By 2003, egg mass densities decline dramatically (i.e., no egg masses found in over 50% of the site) and beetle feeding appeared to decrease (Fig. 10C,F). Percent cover of purple loosestrife at Frontenac Lake was uniform (Fig. 11A). However, total inflorescences were greatly reduced across the south half of the wetland (Fig. 11I). The decrease in egg mass densities from 2002 to 2003 resulted in a rebound in plant height (Fig. 11C-D), total number of inflorescences (Fig. 11E-F), and number of stems (Fig. 11I-J).

Landscape-scale beetle movement. The number of sites visited in each region ranged from 19 sites in Goodhue and Wabasha Counties to 62 sites in the Minnetonka area during the two year study (Table 2). Beetle damage was evident in 85% of the 167 non-release sites visited. Purple loosestrife infestations located in the Minnetonka area had the most damage. Recall, this area also had a greater number of established release sites per km^2 (Table 2). Conversely, the region with the least amount of non-release sites with *Galerucella* spp. beetles present had the fewest established release sites per km^2 (i.e., Goodhue and Wabasha Counties). On average, beetles dispersed 5 km from established release sites to non-release sites within 3 years (Table 2). This trend is consistent between all regions used in the study. The slightly faster colonization of sites within the Minnetonka area (≈ 2 yr) could be attributed to the greater proportion of established release sites compared to non-release sites. *Galerucella* spp. was able to colonize infestations a considerable distance away from established release sites, and the average maximum dispersal distance from all four locations was approximately 19 km.

DISCUSSION

Galerucella spp. disperse and colonize a wetland habitat within 1-2 years after the initial release. In our sites, apparent reductions in purple loosestrife infestations as measured with a variety of plant biometrics in the fall were correlated with high egg mass densities of *Galerucella* spp. observed in the spring. This trend was present in all four wetlands studied. These beetles appear to be well adapted to changing environments and are capable of dispersing and colonizing within large purple loosestrife infestations. This information is important for resource managers in minimizing distribution efforts for controlling purple loosestrife. Although it may take a few years for beetles to distribute themselves across a large wetland, clearly the insects can accomplish this feat without further assistance from resource managers. A recommendation arising from this study would be to select a single location within a wetland to make a release rather than making several smaller releases throughout the wetland, thus minimizing redistribution efforts. As *Galerucella* spp. increase in population, there is a corresponding decrease in purple loosestrife. As a result when loosestrife density declines appreciably the following spring, fewer beetles are produced which in turn releases the plant from herbivory. Plant populations may temporarily rebound as insect pressure declines, but as plant quantity increases there is a concomitant increase in beetle density resulting eventually in an equilibrium where purple loosestrife declines in abundance (Landis et al. 2004). Here we could document small scale (within-wetland) changes in beetle density and plant biometrics.

The ability of *Galerucella* spp. to disperse is not limited to within-wetland movement. *Galerucella* spp. will disperse and locate purple loosestrife infestations over large geographic regions. In particular, beetles were able to find purple loosestrife infestations that were some distance from a known release site and more importantly where there was not a contiguous patch of loosestrife connecting two distant wetlands. These data collected here will enable us to maximize redistribution efforts and we advise resource managers to select wetlands that are greater than 5 km from known release sites for *Galerucella* spp. redistribution. Our analysis demonstrates that, on average, beetles dispersed 5 km from established release sites to non-release sites within 3 years. Because of the constraints of the landscape-scale study, beetles could be moving at a much faster rate than reported, therefore our estimate is likely a conservative prediction of *Galerucella* spp. movement among wetlands. Regardless, *Galerucella* spp. is capable of locating and colonizing new purple loosestrife infestations, thus reducing redistribution efforts from resource managers. This study provides a basic model for assessing the impacts of other potential biological control agents on other invasive species like buckthorn (*Rhamnus cathartica* L.) and garlic mustard (*Alliaria petiolata* [Bieb]).

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Table 1. Semivariograms models, parameters, and r^2 values for all variables used to assess impacts of *Galerucella* spp. movement within wetlands.

Variable	Field site	Year	Model	Nugget[a]	Sill[b]	Range[c]	r^2
Spring Sampling							
Egg masses/stem							
	Frontenac						
		2001	spherical	0.001	0.489	50	0.925
		2002	linear	0.716	1.518	86	0.987
		2003	spherical	0.005	0.454	40	0.922
	Sherburne						
		2001	linear	0.352	0.738	86	0.985
		2002	spherical	0.010	15.750	53	0.985
		2003	linear	1.236	1.236	86	0.894
	Stonebridge						
		2002	spherical	0.178	0.356	79	0.987
		2003	exponential	0.000	0.287	28	0.796
	Wacouta						
		2001	spherical	0.000	0.219	35	0.421
		2002	spherical	0.133	0.345	75	0.946
		2003	exponential	0.170	0.546	72	0.973
% Crown damage							
	Frontenac						
		2001	spherical	0.001	1.875	47	0.729
		2002	linear	2.434	4.584	86	0.991
		2003	spherical	1.34	5.02	69	0.985
	Sherburne						
		2001	linear	0.915	2.096	86	0.958
		2002	spherical	115	1788	31	0.755
		2003	linear	2159.9	215.9	83	0.937
	Stonebridge						
		2002	linear	0.7765	0.7765	87	0.0392
		2003	linear	0.3364	0.3364	87	0.0278
	Wacouta						
		2001	exponential	0.001	1.158	8	0.072
		2002	spherical	0.001	0.958	20	0.000
		2003	spherical	0.065	1.248	20	0.000
Fall Sampling							
% PLS cover							
	Frontenac						
		2002	exponential	0.72	4.39	21	0.964
		2003	spherical	0.01	5.382	48	0.941
	Sherburne						
		2002	exponential	0.357	2.371	32	0.989
		2003	spherical	0.001	1.184	22	0.000
	Stonebridge						
		2002	exponential	0.056	0.6	31	0.997
		2003	spherical	0.053	0.94	49	0.962
	Wacouta						
		2002	exponential	43	997	27	0.999
	Wacouta	2003	spherical	0.001	1.549	47	0.974

Stem height (cm)							
	Frontenac						
		2002	spherical	0.160	10.880	30	0.756
		2003	spherical	6.020	15.340	103	0.999
	Sherburne						
		2002	spherical	1	2464	56	0.967
		2003	linear	2.101	3.613	86	0.852
	Stonebridge						
		2002	exponential	0.000	0.064	37	0.993
		2003	linear	0.417	0.417	87	0.581
	Wacouta						
		2002	spherical	230	1835	164	0.979
	Wacouta	2003	spherical	447	1438	51	0.996
Average inflorescences/m^2							
	Frontenac						
		2002	linear	0.1238	0.1238	86	0
		2003	exponential	0.698	1.815	108	0.967
	Sherburne						
		2002	spherical	0.001	0.94	51	0.935
		2003	spherical	0.0097	0.0664	49	0.967
	Stonebridge						
		2002	spherical	0.258	1.137	59	0.999
		2003	exponential	0.001	1.124	37	0.949
	Wacouta						
		2002	spherical	0.452	0.976	71	0.917
		2003	exponential	0.018	0.724	12	0.572
Total Infloresences/m^2							
	Frontenac						
		2002	linear	1.024	1.024	86	0.537
		2003	exponential	2.800	35.600	39	0.940
	Sherburne						
		2002	spherical	0.001	2.640	56	0.990
		2003	spherical	0.600	3.913	38	0.727
	Stonebridge						
		2002	spherical	0.263	2.970	54	0.996
		2003	exponential	0.250	4.393	38	0.960
	Wacouta						
		2002	spherical	0.819	2.663	64	0.979
		2003	spherical	0.851	2.322	55	0.992
# Stems/m^2							
	Frontenac						
		2002	exponential	0.856	2.614	20	0.768
		2003	spherical	0.010	6.383	80	0.980
	Sherburne						
		2002	exponential	0.094	1.086	17	0.691
		2003	linear	0.736	1.111	86	0.749
	Stonebridge						
		2002	linear	0.386	0.635	87	0.988
		2003	exponential	0.060	0.689	33	0.895
	Wacouta						

	2002	spherical	0.312	0.877	98	0.921
	2003	spherical	0.001	0.970	42	0.956

a--experimental error.
b--sample variance (i.e., measures the degree of similarity between samples).
c--average distance where samples remain correlated spatially.

Table 2. Summarized data from the 2001 and 2002 landscape-scale study.

Location	Area (km^2):	Number of Established Release Sites:	Number of Non-Release Sites Visited:	Mean Dispersal Distance (km) from Release Site ± SE:	Max Dispersal Distance (km) from Release Site:	Number of years to *Galerucella* presence ± SE:	% of Sites Visited with *Galerucella* spp. Present:
Pope and Swift Counties	1,772	11	39	5.5 ± 1.0	20.9	3.2 ± 0.2	72
Minnetonka area[a]	2,129	28	62	2.4 ± 0.4	17.9	2.4 ± 0.1	95
Northeast area[b]	1,154	6	47	7.0 ± 0.7	20.4	2.8 ± 0.2	89
Goodhue and Wabasha Counties	825	3	19	4.1 ± 1.1	17.8	3.8 ± 0.2	69
Total	5,880	48	167	4.7 ± 0.4	19.3 ± 8.2	2.8 ± 0.1	85

[a] includes Wright, Hennepin, and Carver Counties.
[b] includes Anoka, Chisago, Ramsey, and Washington Counties.

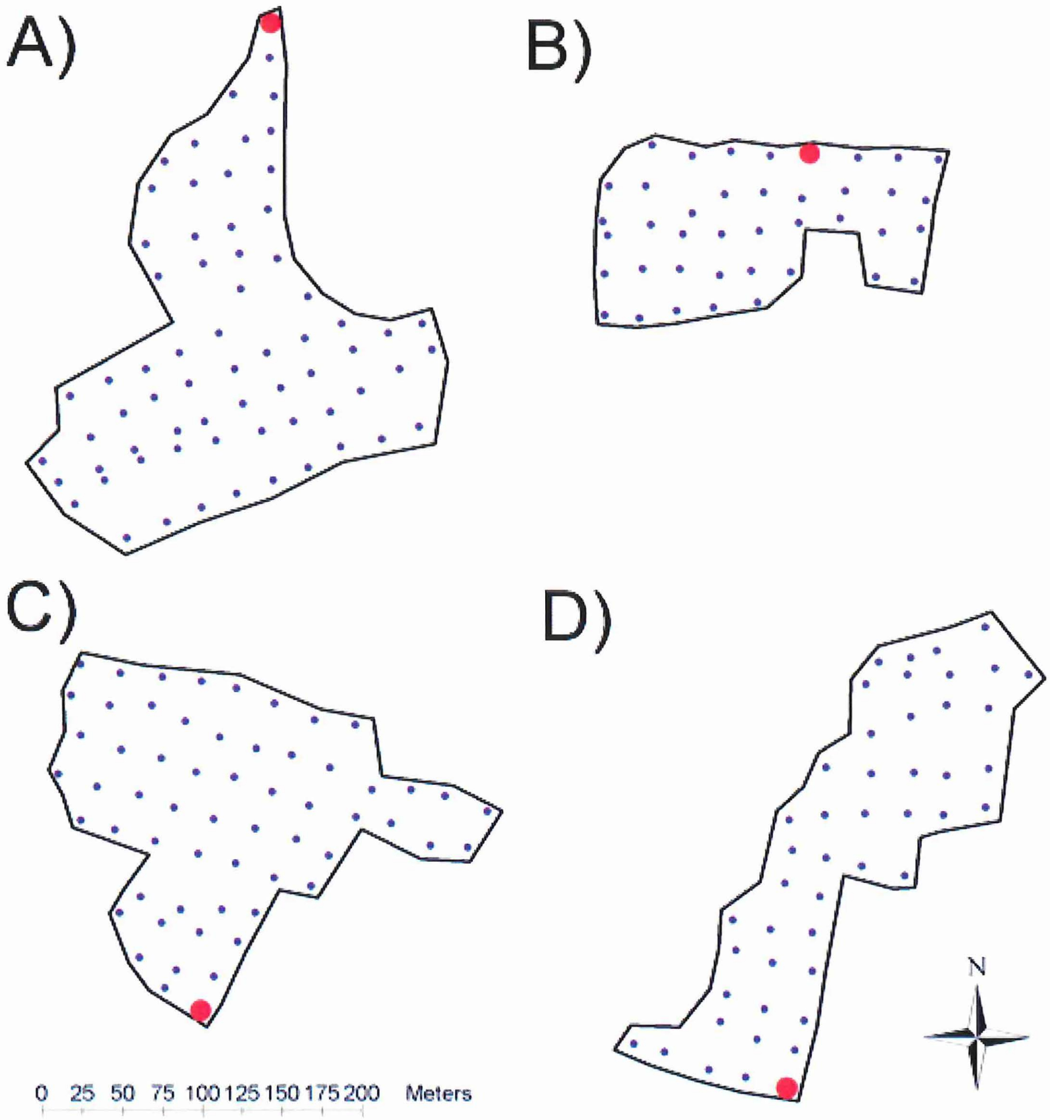

Figure 1. Map of waypoints and release points (●) for all four wetlands used in the within-wetland study of *Galerucella* spp. beetle movement. A) Wacouta Pond, B) Sherburne Pool, C) Stonebridge Road, and D) Frontenac Lake.

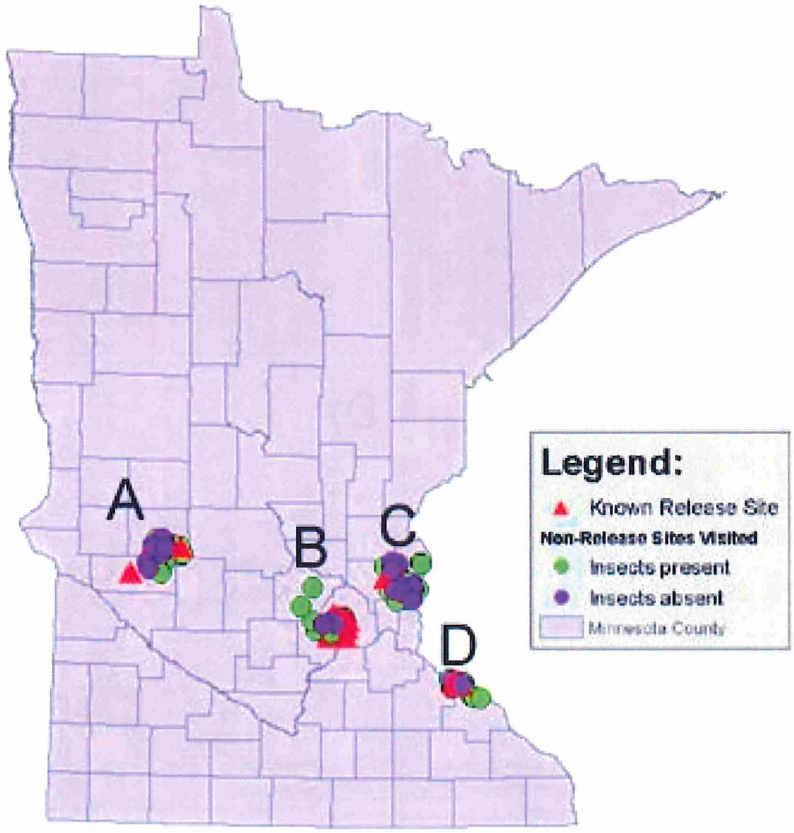

Figure 2. Map of four geographic regions used in the landscape-scale movement study. Areas included: A) Pope and Swift Counties, B) Wright, Hennepin, and Carver Counties referred to as Minnetonka area, C) Anoka, Chisago, Ramsey, and Washington Counties referred to as Northeast area, and D) Goodhue and Wabasha Counties.

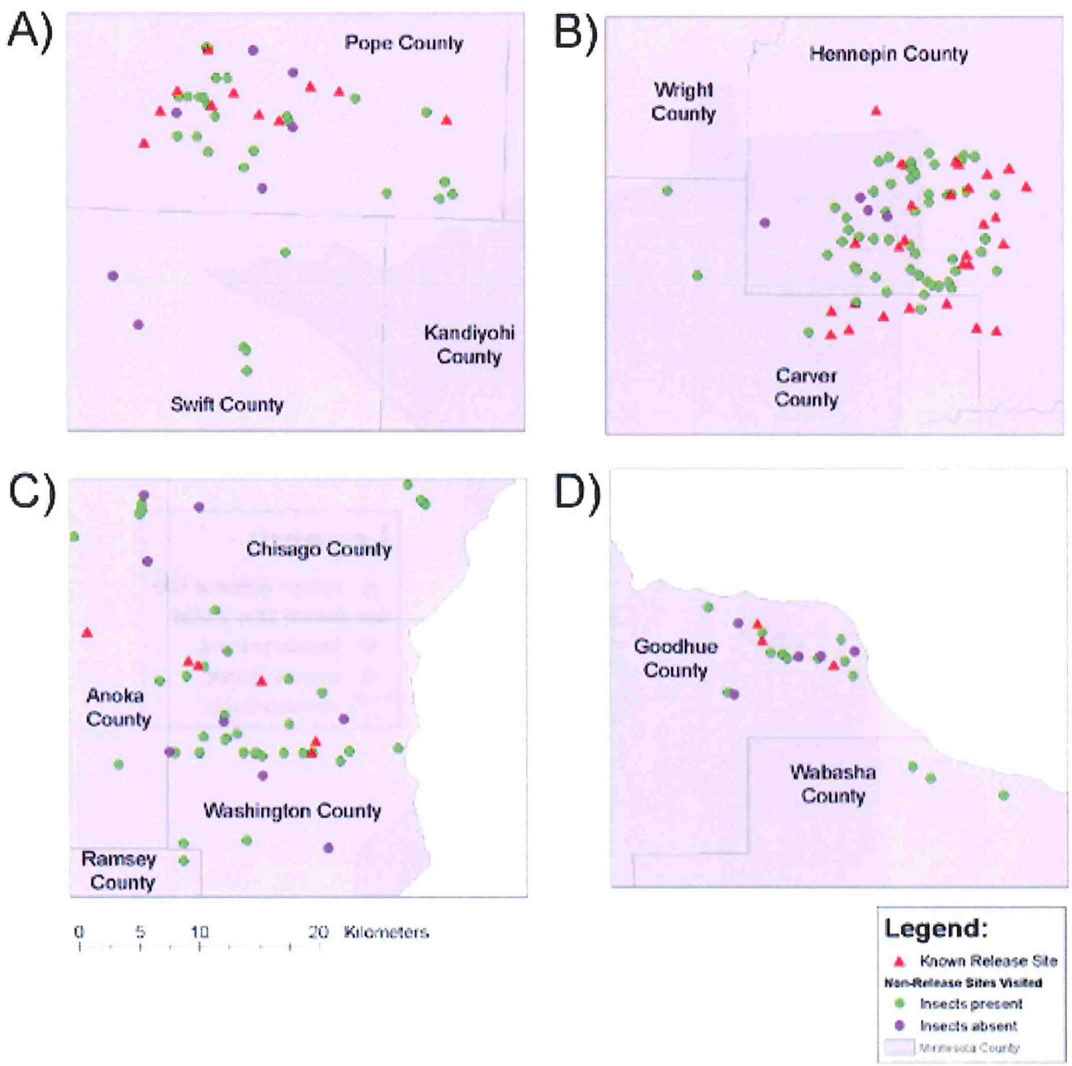

Figure 3. Distribution of purple loosestrife infested wetlands visited in 2001 and 2002. *Galerucella* spp. presence/absence was noted in all areas visited. Areas included: A) Pope and Swift Counties, B) Wright, Hennepin, and Carver Counties referred to as Minnetonka area, C) Anoka, Chisago, Ramsey, and Washington Counties referred to as Northeast area, and D) Goodhue and Wabasha Counties.

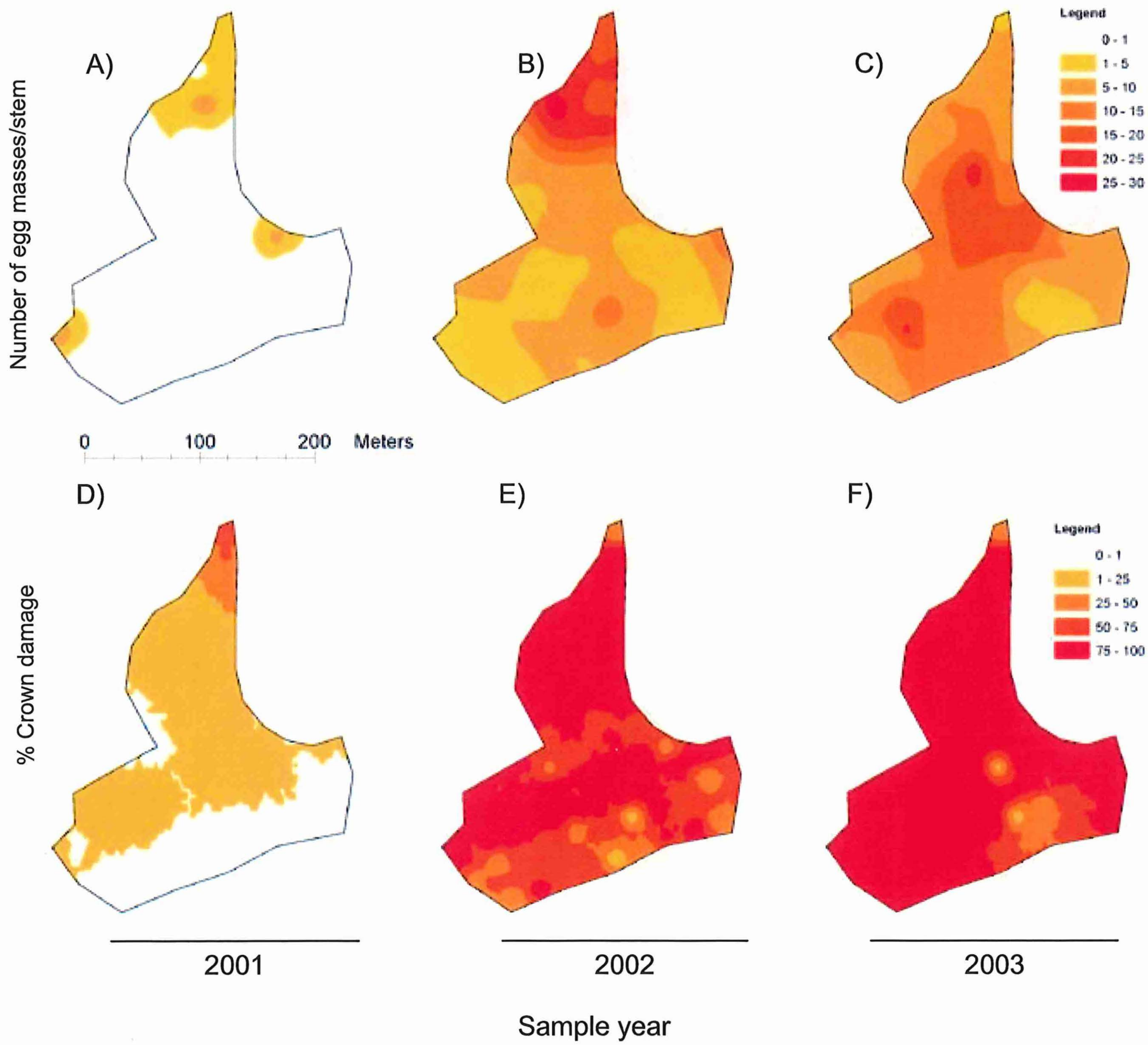

Figure 4. Contour maps showing interpolated surfaces of egg mass densities measured in A) 2001, B) 2002, and C) 2003 and percent purple loosestrife crown damage observed in D) 2001, E) 2002, and F) 2003 at Wacouta Pond.

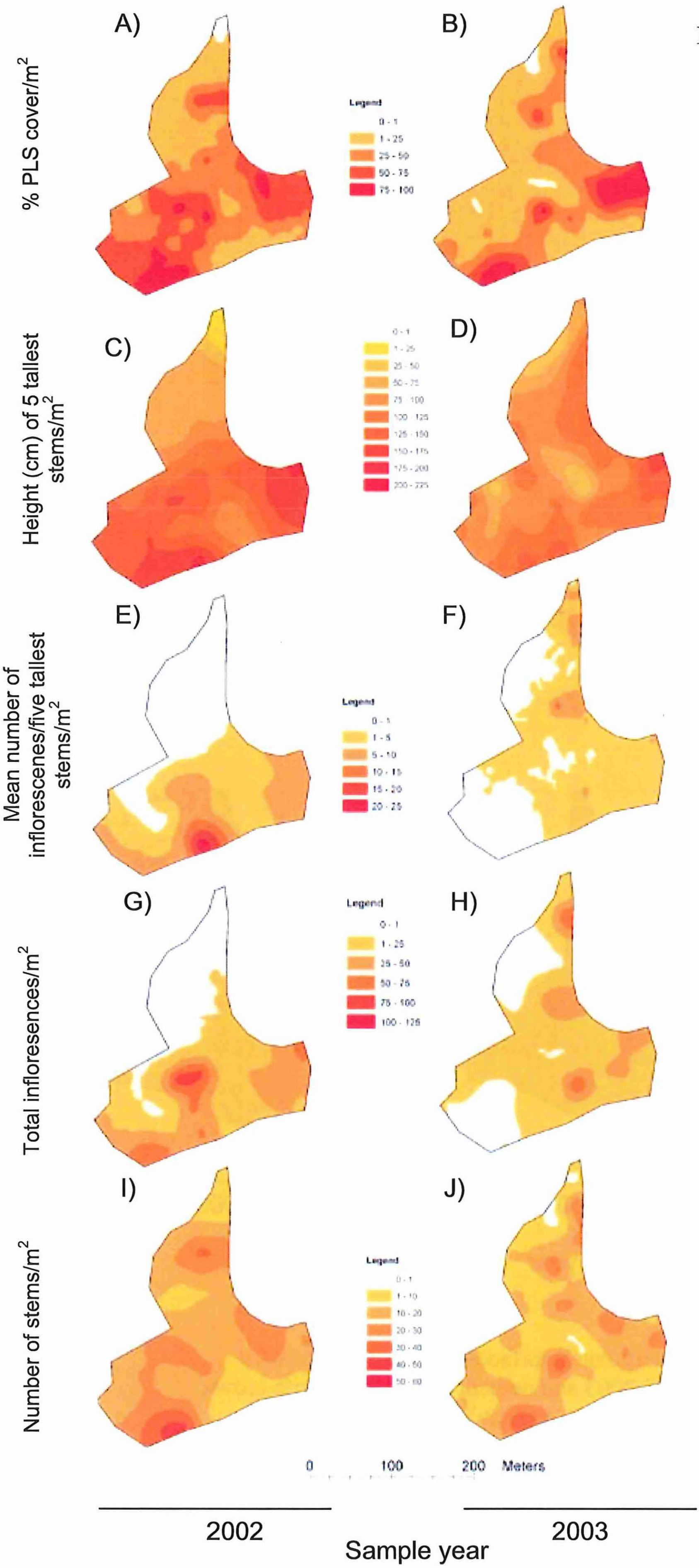

Figure 5. Contour maps showing interpolated surfaces for percent purple loosestrife (PLS) cover per m^2 (A, B), height (cm) of the five tallest stems per m^2 (C, D), mean number of inflorescences of per five tallest stems per m^2 (E, F), total inflorescences per m^2 (G, H), and number of stems per m^2 (I, J) at Wacouta Pond in 2002 and 2003, respectively.

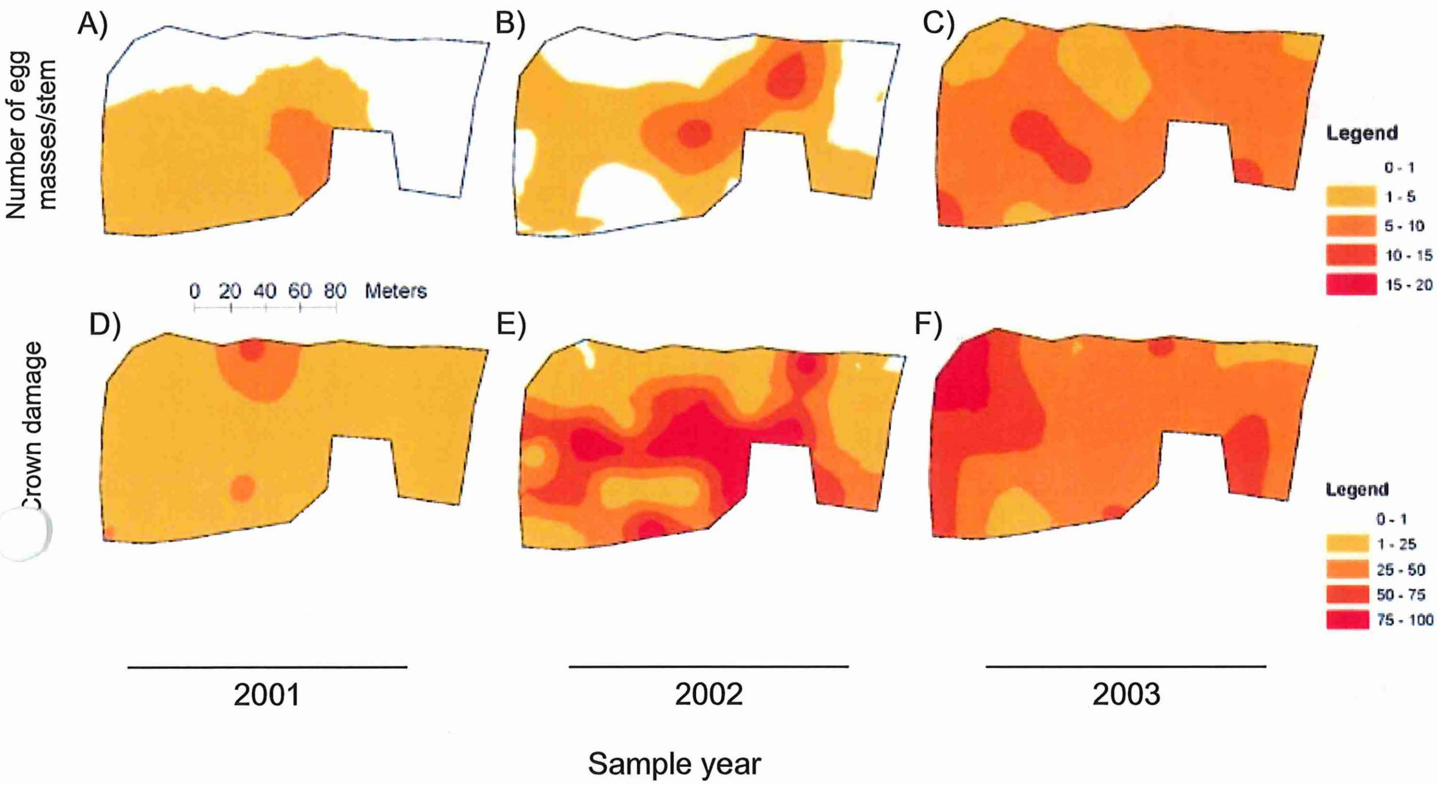

Figure 6. Contour maps showing interpolated surfaces of egg mass densities measured in A) 2001, B) 2002, and C) 2003 and percent purple loosestrife crown damage observed in D) 2001, E) 2002, and F) 2003 at Sherburne Pool.

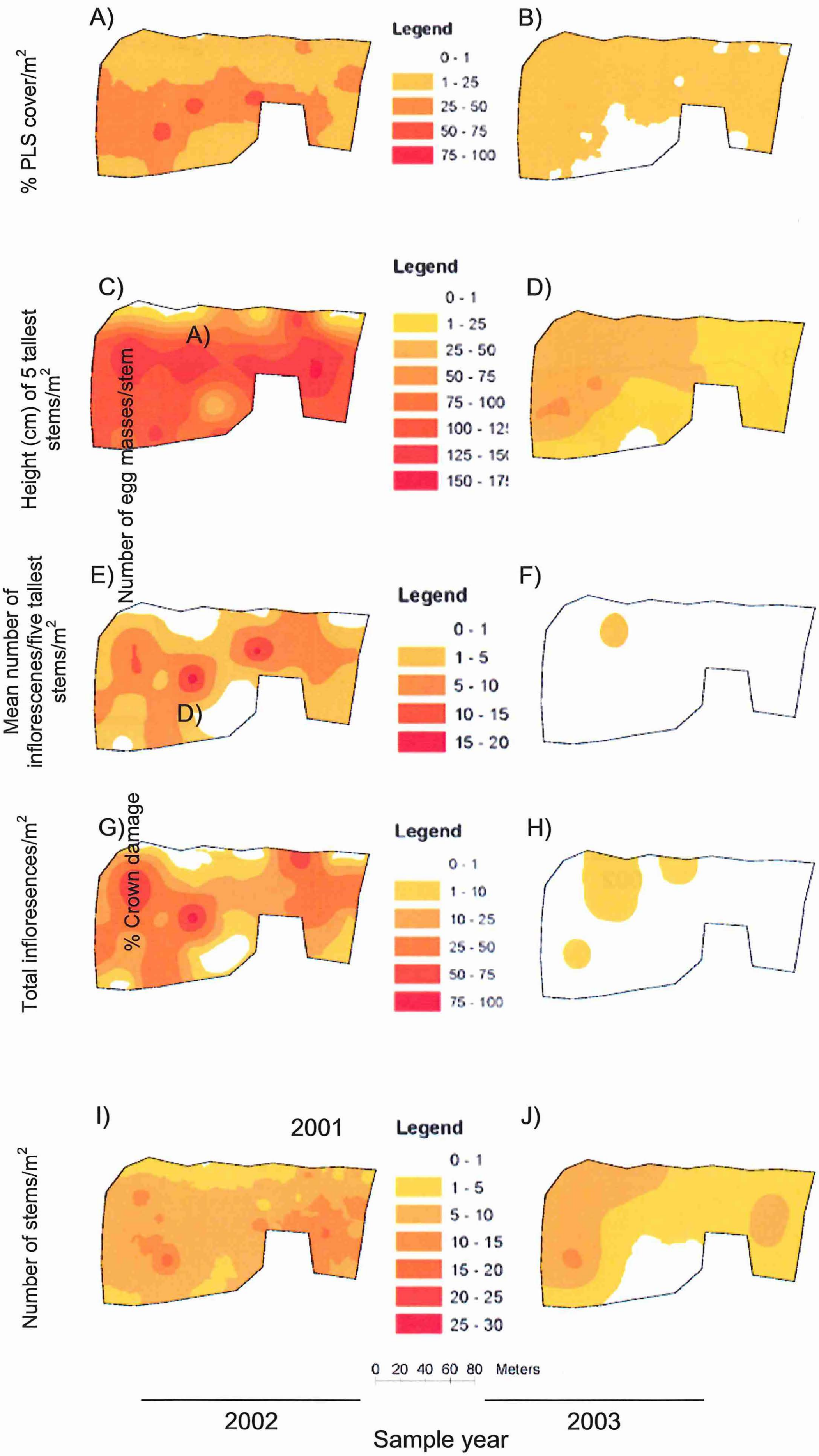

Figure 7. Contour maps showing interpolated surfaces for percent purple loosestrife (PLS) cover per m^2 (A, B), height (cm) of the five tallest stems per m^2 (C, D), mean number of inflorescences of per five tallest stems per m^2 (E, F), total inflorescences per m^2 (G, H), and number of stems per m^2 (I, J) at Sherburne Pool in 2002 and 2003, respectively.

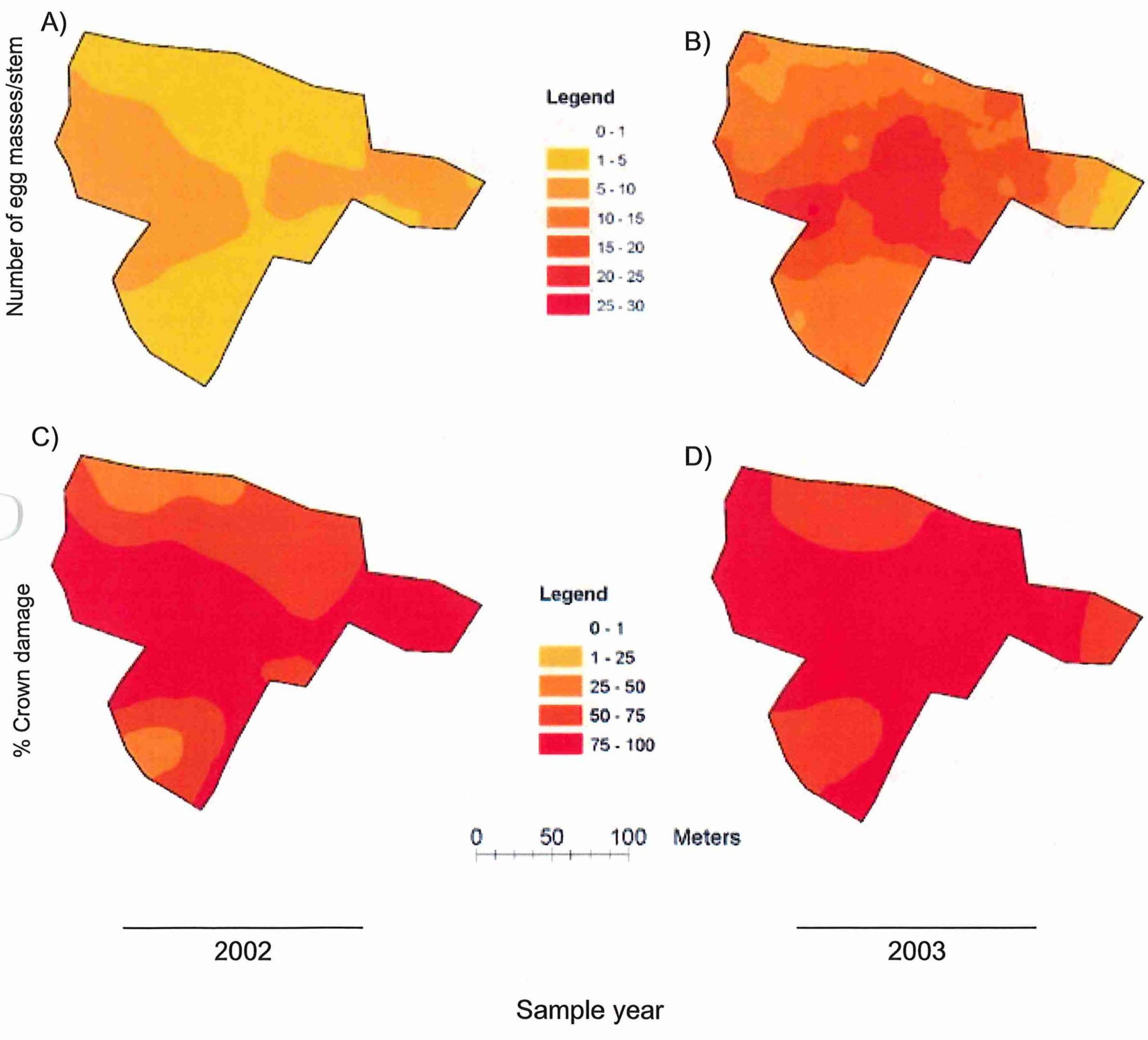

Figure 8. Contour maps showing interpolated surfaces of egg mass densities measured in A) 2002 and B) 2003, and percent purple loosestrife crown damage observed in C) 2002 and D) 2003 at Stonebridge Road.

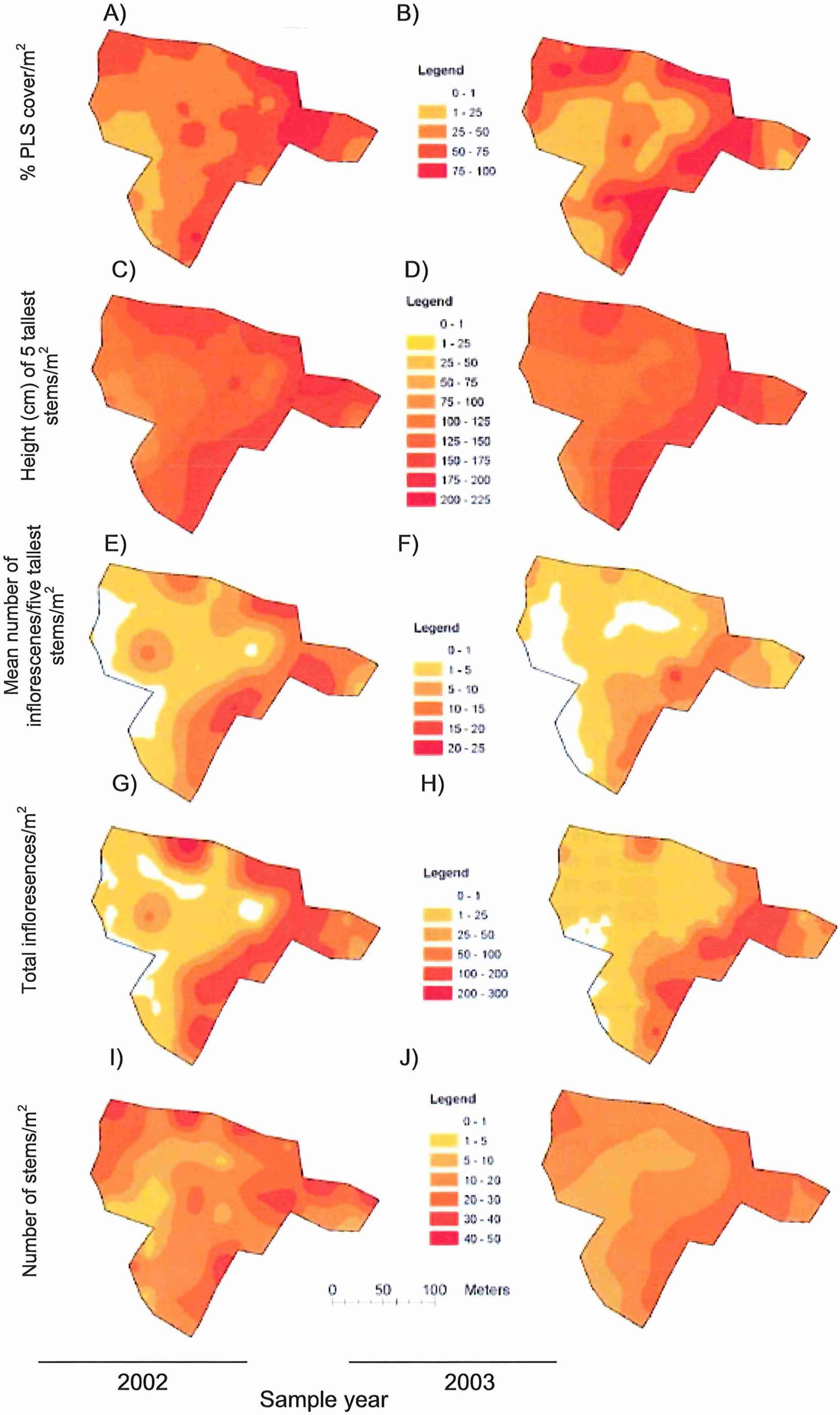

Figure 9. Contour maps showing interpolated surfaces for percent purple loosestrife (PLS) cover per m^2 (A, B), height (cm) of the five tallest stems per m^2 (C, D), mean number of inflorescences of per five tallest stems per m^2 (E, F), total inflorescences per m^2 (G, H), and number of stems per m^2 (I, J) at Stonebridge Road in 2002 and 2003, respectively.

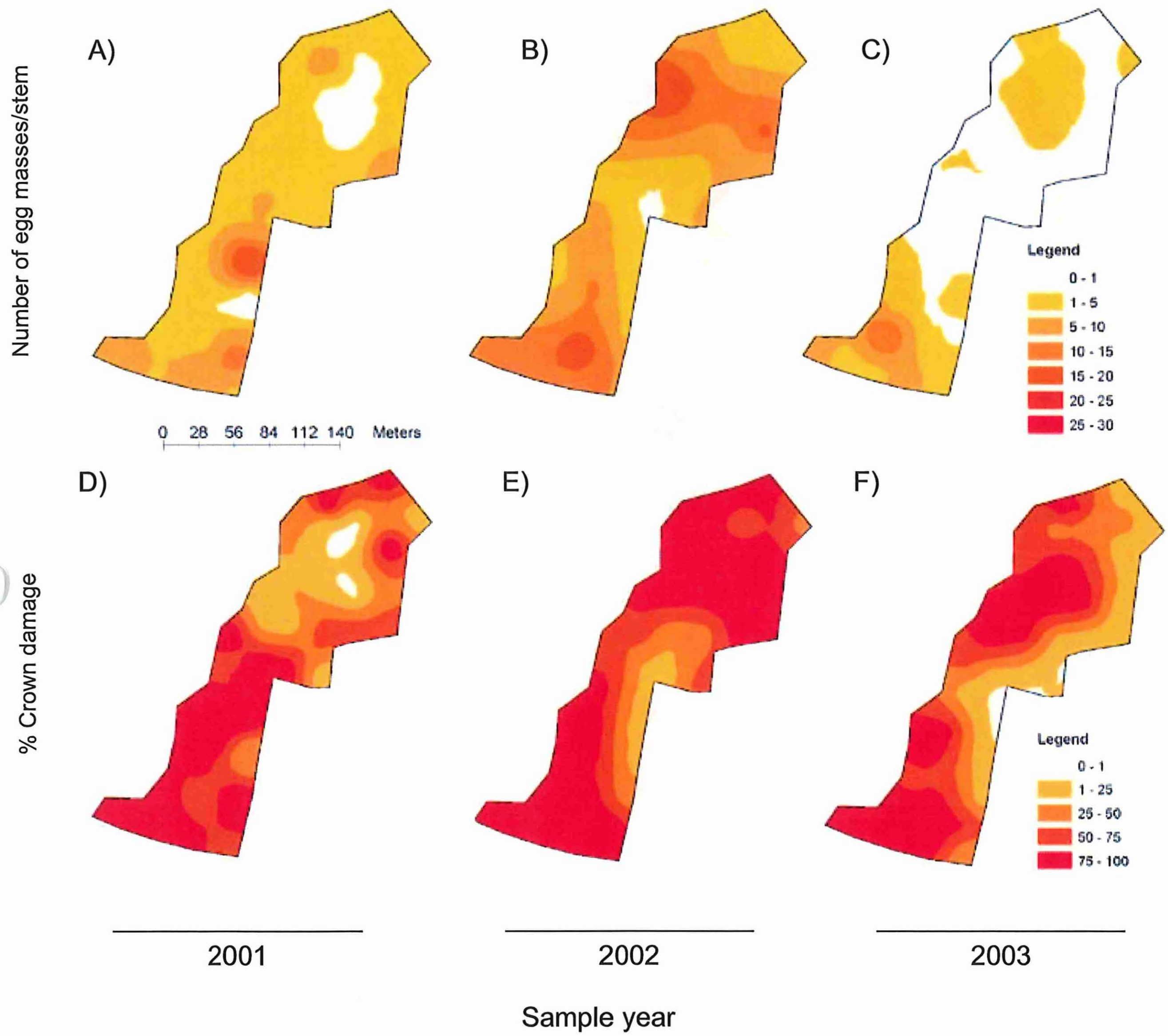

Figure 10. Contour maps showing interpolated surfaces of egg mass densities measured in A) 2001, B) 2002, and C) 2003 and percent purple loosestrife crown damage observed in D) 2001, E) 2002, and F) 2003 at Frontenac Lake.

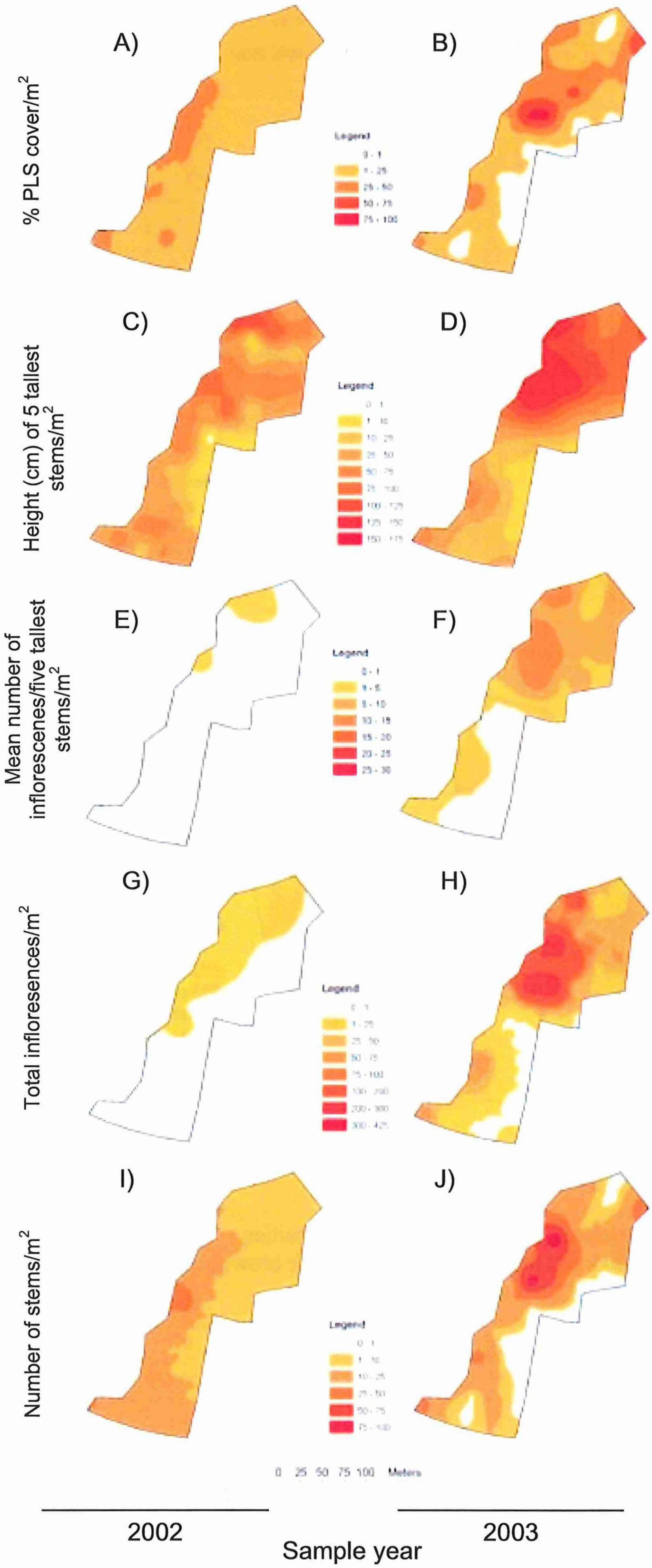

Figure 11. Contour maps showing interpolated surfaces for percent purple loosestrife (PLS) cover per m^2 (A, B), height (cm) of the five tallest stems per m^2 (C, D), mean number of inflorescences of per five tallest stems per m^2 (E, F), total inflorescences per m^2 (G, H), and number of stems per m^2 (I, J) at Frontenac Lake in 2002 and 2003, respectively.